Case Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research

48(4) 1–7

DOI: 10.1177/0300060519894120
journals.sagepub.com/home/imr

SAGE

Fatal rare case of measles complicated by bilateral pulmonary embolism: a case report and short literature review

Simona Claudia Cambrea[1,*], Adriana Luminita Balasa[2,*], Oana Cristina Arghir[3] and Cristina Maria Mihai[2]

Abstract

Different endemic outbreaks of measles have been diagnosed worldwide during the last several years. Some have had severe and fatal complications, possibly because of decreasing vaccination rates. The present case report describes an unvaccinated boy aged 2 years 11 months who was diagnosed with severe measles complicated by pulmonary embolism (PE). Clinical examination revealed a maculopapular rash, hyperemic pharynx, Koplik's spots, upper respiratory airway obstruction, and tachycardia with no meningeal signs of irritation. Laboratory investigations showed leukocytosis, anemia, normal liver enzyme levels, a moderately high C-reactive protein level (26 mg/L), a high erythrocyte sedimentation rate (65 mm/h), and immunoglobulin M positivity for measles. The patient was treated with antibiotic therapy (meropenem at 20 mg/kg every8 hours) and supportive measures (anti-inflammatory drugs and intravenous rehydration). On the fourth day of hospitalization, the patient's general condition became profoundly altered; although cardiorespiratory resuscitation maneuvers were initiated, the child died. Autopsy revealed bilat- eral pleural effusion with serous citrine fluid, acute purulent bronchopneumonia, bilateral hilar adenopathy, and bilateral PE. Additional research is needed to establish optimal care for pediatric patients with measles, especially when complicated by PE.

[1]Infectious Diseases Department, Faculty of Medicine, Ovidius University of Constanta, Romania
[2]Pediatry Department, Faculty of Medicine, Ovidius University of Constanta, Romania
[3]Pulmonology Department, Faculty of Medicine, OvidiusUniversity of Constanta, Romania

*These authors contributed equally to this work.

Corresponding author:
Oana Cristina Arghir, Pulmonology Department, Facultyof Medicine, Ovidius University of Constanta, 1 University Alley, Campus, Corp B, Constanta 900470, Romania.
Email: arghir_oana@yahoo.com

Keywords
Measles, pulmonary thromboembolism, hilar adenopathy, vaccination, treatment, pediatric

Date received: 23 May 2019; accepted: 20 November 2019

Introduction

The incidence of measles virus infection in childhood has dramatically increased during the last several years.[1] This increase has been associated with more complica- tions of the disease and even increased mor- tality.[2] Pulmonary embolism (PE) remains a rare complication of measles. Although it was first described many decades ago by von Löschner,[3] our understanding of pedi- atric PE remains unclear. This could be due to the neglect or rarity of pediatric PE diagnosis, limiting the development of a standardized approach to its management. Therefore, the incidence of undiagnosed PEas a complication in children with various infectious diseases might be underesti- mated, resulting in increased morbidity and especially mortality of measles.[4]

We here in report a case of a child with confirmed measles complicated by PE who was admitted to a hospital specializing in infectious diseases. The patient's parents provided informed consent, and the ethics committee of Constanta Clinical Infectious Diseases Hospital approved the publication of this case report.

Case report

A boy aged 2 years 11 months was admittedto the Pediatric Department of ConstantaClinical Infectious Diseases Hospital in Romania. The child had been symptomatic for the last several days and was referred from the emergency unit to our hospital by ambulance because of an altered general condition. The child presented with a fever (38.8°C), loss of appetite, dry tongue, gen- eralized rash, mucopurulent rhinorrhea, cough, dyspnea with intercostal retraction,fine crackles, rhonchus, rhythmic heart sounds, a supple and mobile abdomen with respiratory movements, gurgling, accelerated intestinal transit, four watery stools per day, and normal urination. Additionally, a maculopapular rash on the face, upper limbs, and trunk had been observed by his parents during the last 48 hours. At admission, general clinical examination revealed a hyperemic pharynx, Koplik's pathognomonic spots, an extend- ed maculopapular rash, dry cough, dys- pnea, intercostal and subcostal retractions, fatigue, fine crackles, rhonchus at lung aus- cultation, tachycardia (155 beats/minute), and a Glasgow score of 15 with no menin- geal signs of irritation. The child's epidemi-ological history revealed the absence of any previous vaccination, including vaccination to measles.

Laboratory tests indicated positive serol- ogy for measles (i.e., immunoglobulin M), leukocytosis (27.3 10^9 cells/L; referencerange, 6–17 10^9 cells/L), anemia, a moder-ately high C-reactive protein level (26 mg/L; reference range, 0–5 mg/L), and high eryth- rocyte sedimentation rate (65 mm/hour; reference range, 3–9 mm/hour). The levels of liver enzymes (alanine aminotransferase and aspartate aminotransferase) and creati- nine were normal. Other laboratory investi- gations revealed a negative stool culture, thepresence of *Staphylococcus epidermidis* in both right and left conjunctival secretions, and no Gram-positive or -negative cocci in the pharyngeal exudate. During hospitaliza- tion, the child's altered clinical condition persisted, with a decrease in the arterial oxygen saturation from 97% to 90%,

crackles, tachycardia, and diuresis; his stools remained normal. Treatment included antipyretic, analgesic, anti-inflammatory, and antitussive drugs; intravenous rehydrationwith electrolyte rebalancing and energy support; antibiotic and antifungal therapy; immunoglobulin to stimulate the immune system and prevent severe complications; and supplemental oxygen therapy. Given the leukocytosis and elevated C-reactive protein level, a bacterial superinfection with probable pulmonary involvement was considered, and meropenem was recommended as a broad-spectrum antibiotic. The patient thus received meropenem at 20 mg/kg every 8 hours. Despite slow improvement during the first 3 days of hospitalization with only slightly low arterial oxygen saturation (around 92%), the patient subsequently developed bradycardia (65 beats/minute) with more frequent episodes of oxygen desaturation under continuous oxygen supplementation. During the fourth day of hospitalization, the child developed sudden-onset cardiopulmonary gasping with metabolic acidosis and severe bradycardia (40 beats/minute). Cardiorespiratory resuscitation maneuvers were initiated for morethan 1 hour, including patient repositioning, secretion aspiration, continuous-flow oxygen, corticosteroids, adrenaline, and external cardiac massage; however, the child died.

At autopsy, a macroscopic anatomical pathological examination revealed cerebral edema, internal hydrocephalus, hemorrhage of the choroid plexuses, and enlargement of the cerebral tonsils (Figure 1). We also observed polyserositis with acute pericarditis,

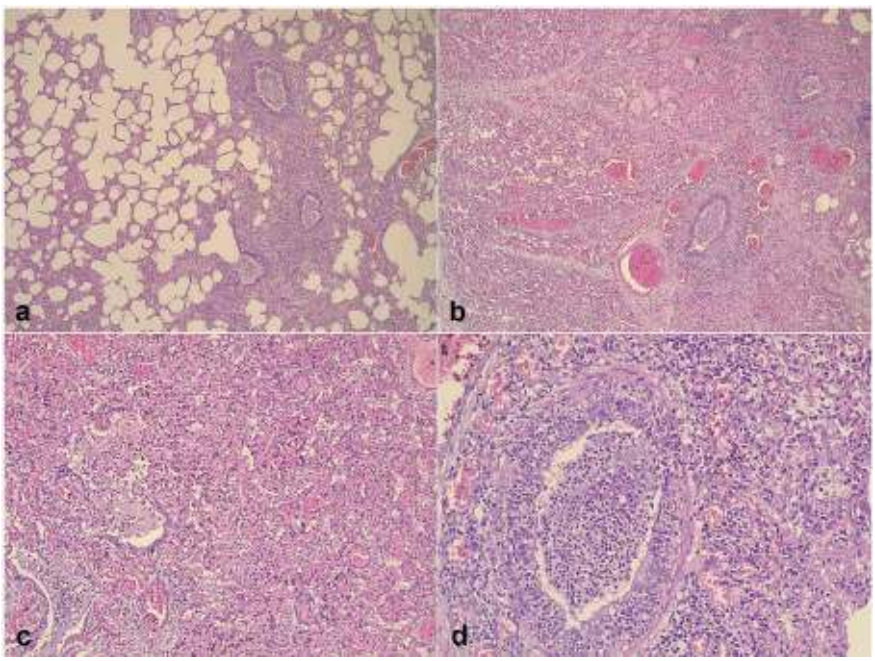


Figure 1. (a) Disseminated bronchopneumonia: focus of inflammatory condensation surrounding bronchioles with intraluminal suppurative exudate and bronchiolitis [hematoxylin–eosin (HE) stain, 10×]. (b) Inflammatory condensation surrounding a bronchiole with intraluminal suppurative exudate and vascularcongestion (HE stain, ×10). (c) Leukocytic alveolitis with vascular and septal congestion; partial disruption ofbronchiole walls by inflammation (HE stain, ×20). (d) Focus of inflammatory condensation surrounding a bronchiole with intraluminal suppurative exudate (HE stain, ×40).

bilateral peritonitis and pleural effusion with serous citrine fluid, acute purulent broncho-pneumonia, hilar adenopathy, and bilateral pulmonary thromboembolism (Figures 2 and 3) as well as stasis at the level of the liver, spleen, and kidney.

Discussion

The measles virus is an RNA virus that belongs to the Paramyxoviridae family.Its incubation period ranges from 10 to 12 days. Affected patients initially develop signs of an upper respiratory tract infection lasting about 2 to 4 days, followed by patho- gnomonic Koplik's spots and a generalized maculopapular rash.[5,6] The measles virus is contagious, and its transmission rate is high (90%).[7,8] In contrast, in populations with higher vaccination rates, acute measles infection is most often caused by infections acquired abroad.[9]

An embolus is a "travelling clot," and PEis usually a complication of deep vein thrombosis. In children, PE is a very rare and unrecognized condition, but it is usual- ly fatal.[10] In autopsy examinations of chil- dren with PE, the intravitam diagnosis could not be established.[11] Although many physicians consider PE to be silent in chil- dren, symptoms and signs are seen but ignored by clinicians in some cases, leading to a misdiagnosis of pneumonia, malignan- cy, or exacerbation of heart failure.[12,13]

Aspirin is used to prevent PE in adults, but this advantage is lacking in children.

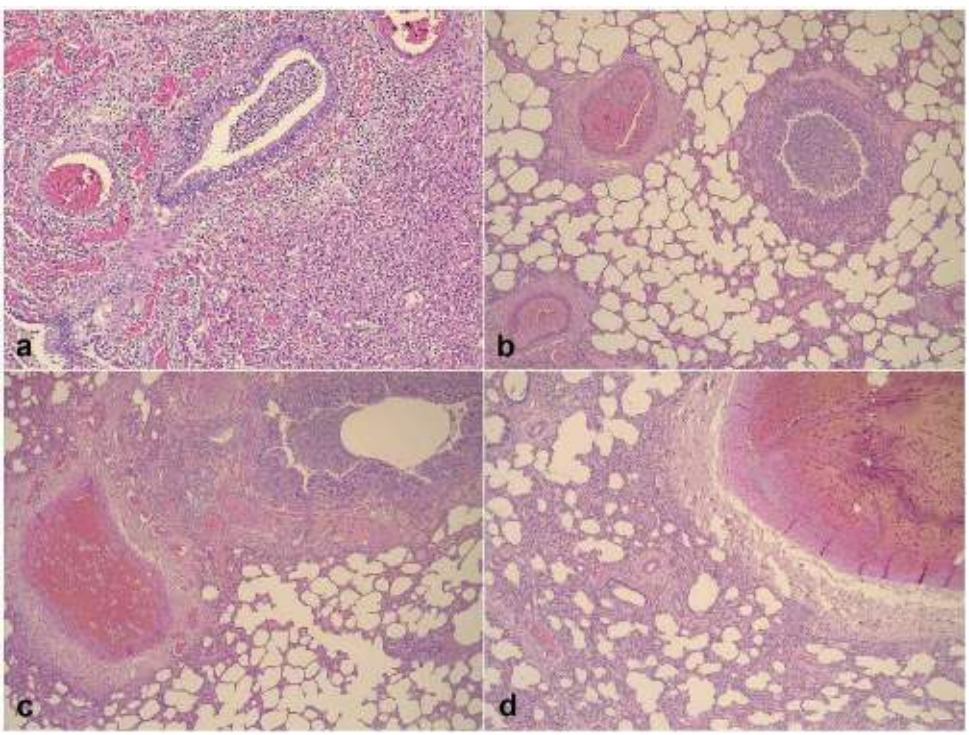


Figure 2. (a) Inflammatory condensation surrounding a bronchiole with intraluminal suppurative exudate and vascular congestion [hematoxylin–eosin (HE) stain, ×10]. (b) Exudative bronchiolitis and intravascular red thrombi (HE stain, ×10). (c) Exudative bronchitis and intra-arterial red thrombus (HE stain, ×10).
(d) Intra-arterial mixed thrombus (HE stain, ×10).

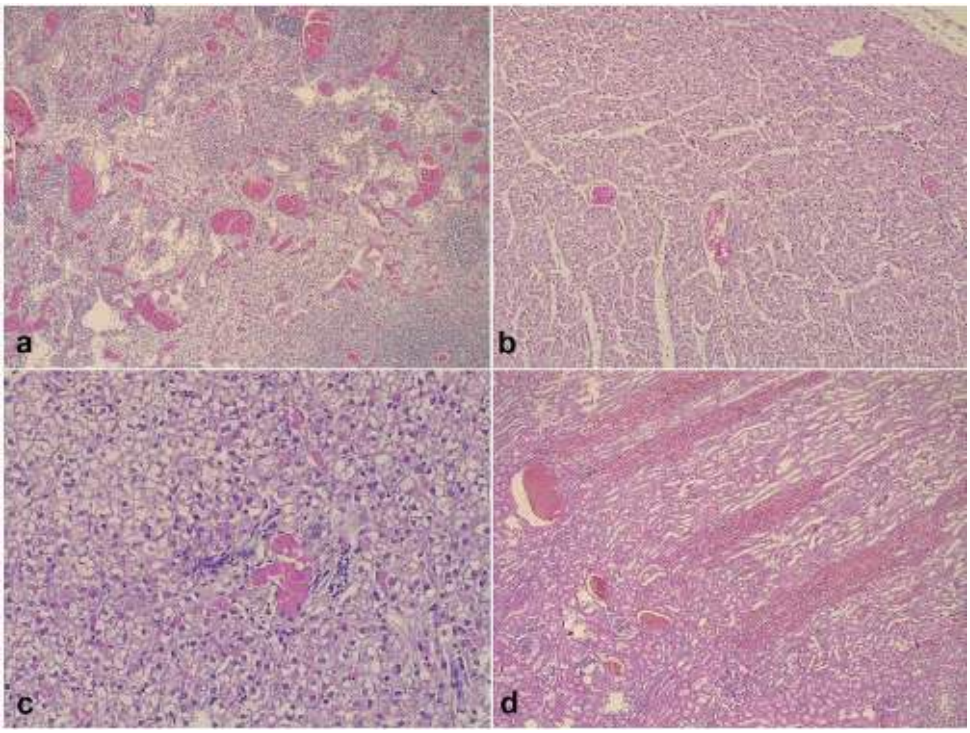


Figure 3. (a) Lymph node with marked vascular congestion and lymphocyte depletion [hematoxylin–eosin(HE) stain, ×10]. (b) Heart with minimal vascular congestion and interstitial edema (HE stain, ×10). (c) Liverwith portal and sinusoidal congestion (HE stain, ×20). (d) Kidney with vascular, peritubular capillary, and glomerular congestion (HE stain, ×10).

Aspirin is not usually used to treat fever in children because it has been associated with a high risk of Reye syndrome in the presence of certain viral illnesses.[5] One study showed that thromboembolic events can be a common cause of mortality in hospitalized children without surgical interventions.[14] Because our patient's respiratory condition continuously deteriorated, rapid diagnosis was crucial for survival. Complications of measles usually occur in children aged <5 years and adults aged >20 years.[15]

PE in children may have classic symptomatology[16]; unlike in adults, however, its onset is more silent.[4] Delayed diagnosis of PE may result from misdiagnosis or confusion with pneumonia or exacerbation of heart failure.[16] In many cases, only autopsy revealed the PE; no antemortem diagnosis of PE had been made.[17] Leukocytosis in a child with a viral infection, as in our patient (17.4×10^9 cells/L; reference range, $6–12 \times 10^9$ cells/L), can be a surrogate biomarker of acute PE. Thus, maintaining a high index of suspicion for PE in children is mandatory if symptoms such as chest pain, hemoptysis, fever, or even syncope are present. Moreover, tachycardia and edema due to deep vein thrombosis may be present.[18] Therefore, many medical conditions and infections can mask the diagnosis of PE, leading to delayed diagnosis or misdiagnosis of PE with development of subsequent complications.[19] For

example, our patient did not exhibit tachycardia but instead showed bradycardia, which was related to the presence of peri- carditis. However, many epidemiological studies are based on autopsy findings mostly in children who are already known to have PE.[20] In another review of 3600 pediatric autopsies, Buck et al.[21] reported a 3.7% incidence of massive PE; amongthese patients, 31% died.

Most importantly, every physician must be aware of the beneficial effects of a strict vaccination policy, which is mandatory to prevent further infections and eliminate sporadic cases or epidemics of measles in the future.[7] Based on these observations, we further recommend prospective clinical trials specifically based on the risk catego- rization of pediatric PE.

The most common adverse reactions observed after measles vaccination are fever and rash.[22] Severe adverse effects, the frequency of which is unknown, include subacute sclerosing panencephalitis, febrile seizures, acute encephalitis, Guillain–Barré syndrome, and Stevens–Johnson syndrome, but not PE.[22]

In conclusion, children with measles should be considered to be at risk of PE. Although PE in children is a rare medical condition and may often go unrecognized, it can potentially be fatal. Therefore, additional research is needed to establish optimal management of measles in children, especially when complicated by PE.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD

Simona Claudia Cambrea https://orcid.org/0000-0003-4898-8938

References

1. Solomon T, Michael BD, Smith PE, et al. Management of suspected viral encephalitis in adults-Association of British Neurologists and British Infection Association National Guidelines. *J Infect* 2012; 64: 347–373.
2. World Health Organization. Global control and regional elimination of measles, 2000–2012. *Wkly Epidemiolog Rec* 2014; 89: 45–52.
3. von Löschner JWF. Phlebitis venae cruralis sinistrae, peri-et myocarditis, embolia et oedema pulmonum. *Jahrb f Kenderh* 1861; 4: 6.
4. Brandao LR, Labarque V, Diab Y, et al. Pulmonary embolism in children. *Semin Thromb Hemost* 2011; 37: 772–785.
5. Mulholland EK, Griffiths UK and Biellik R. Measles in the 21st century. *N Engl J Med* 2012; 366: 1755–1757.
6. Sabella C. Measles: not just a childhood rash. *Cleve Clin J Med* 2010; 77: 207–213.
7. [No authors listed]. Measles vaccines: WHO position paper. *Wkly Epidemiol Rec* 2009; 84: 349–360.
8. Stokes J Jr, Reilly CM, Buynak EB, et al. Immunologic studies of measles. *Am J Hyg* 1961; 74: 293–303.
9. Centers for Disease Control and Prevention (CDC). Measles-United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012; 61: 253–257.
10. Zaidi AU, Hutchins KK and Rajpurkar M. Pulmonary embolism in children. *FrontPediatr* 2017; 5: 170.
11. Buck JR, Connors RH, Coon WW, et al. Pulmonary embolism in children. *J Pediatr Surg* 1981; 16: 385–391.
12. Biss TT, Brandao LR, Kahr WH, et al. Clinical features and outcome of pulmonary embolism in children. *Br J Haematol* 2008; 142: 808–818.
13. Dijk FN, Curtin J, Lord D, et al. Pulmonary embolism in children. *Paediatr Respir Rev* 2012; 13: 112–122.

14. Patocka C and Nemeth J. Pulmonary embolism in pediatrics. *J Emerg Med* 2012; 42: 105–116.
15. Perry RT and Halsey NA. The clinical significance of measles: a review. *J Infect Dis* 2004; 189(Suppl 1): S4–S16. 10.1086/377712
16. Smellie JM. Pulmonary embolism in childhood. *Arch Dis Child* 1929; 4: 328–329.
17. Rajpurkar M, Warrier I, Chitlur M, et al. Pulmonary embolism-experience at a single children's hospital. *Thromb Res* 2007; 119: 699–703.
18. Bernstein D, Coupey S, Schonberg SK. Pulmonary embolism in adolescents. *Am J Dis Child* 1986; 140: 667–671.
19. Goldsby RE, Saulys AJ and Helton JG. Pediatric pulmonary artery thromboembolism: an illustrative case. *Pediatr Emerg Care* 1996; 12: 105–107.
20. Patocka C and Nemeth J. Pulmonary embolism in pediatrics. *J Emerg Med* 2012; 42: 105–116.
21. Buck JR, Connors RH, Coon WW, et al. Pulmonary embolism in children. *J Pediatr Surg* 1981; 16: 385–391.
22. Davies EG, Elliman DAC, Hart CA, et al. Measles in *Manual of Childhood Infections*. *Saunders* 2001; 345–349.

Journal of Environmental Protection and Ecology 20, No 4, 2087–2092 (2019)

Public health – environmental medicine

NITRATE POISONING IN INFANTS FROM DOBROGEA –ROMANIA

C. M. MIHAI[a], T. CHISNOIU[a], S. CHIRILA[b]*, AL. BALASA[c], S. C. CAMBREA[d], D. M. ILIESCU[e]

[a]*Clinic of Pediatry, Faculty of Medicine, 'Ovidius' University, 1 Aleea Universitatii, Campus Corp B, Constanta, Romania*
[b]*Medical Informatics and Biostatistics, Faculty of Medicine, 'Ovidius' University, Constanta, Romania*
E-mail: sergiu.chirila@univ-ovidius.ro
[c]*Pediatric Department, Clinical County Emergency Hospital of Constanta,145 Tomis Avenue, 900 591 Constanta Romania*
[d]*Infectious Diseases Clinic, Faculty of Medicine, 'Ovidius' University,100 Ferdinand Avenue, 900 178 Constanta, Romania*
[e]*Faculty of Medicine, 'Ovidius' University, 1 Aleea Universitatii, Campus Corp B, Constanta, Romania*

Abstract. Infants for whom the tea or vegetables are prepared with water still remain a high-risk group for nitrate poisoning. A retrospective study between 2014–2019 was conducted on 23 patients (under 8 months old), diagnosed in the Pediatric Department of the Clinical County Emergency Hospital of Constanta with methemoglobinemia due to nitrate poisoning. Clinical examination data like medi- cal history, symptoms, gender, and weight and food consumption were collected. Gasometry (i.e. pO_2, pCO_2, and pH) and nitrituria were also recorded. About 52.17% had methemoglobin (MetHb) between 10–31%, 34.78% had MetHb between 31–60% and 13.04% had MetHb between 61–70%. Out of the 12 girls and 11 boys, tachycardia was present only at the patients who presented MetHb between 61 and 70%. The weight of the infants varied between 8550 and 9900 g and all were from rural areas. On the day of admission, infants under 3 months were fed with milk formula (82.62%), those between 3–6 months received tea and milk formula (13.04%) and those at more than 6 months received puree of mixed vegetables (4.34%). Partial pressure of oxygen (pO_2) seems to have slightly increased values in contrast with pCO_2, a normal pH level and the presence of nitrituria in all infants. Vitamin C administered intravenously was used in all cases as treatment. In order to prevent nitrate poisoning, public health measures must be implemented at the population level.

Keywords: poisoning, nitrate, methemoglobinemia, infant food.

AIMS AND BACKGROUND

Methemoglobinemia or blue baby syndrome is a rare cause of cyanosis in pedi- atric patients due to nitrate poisoning. Methemoglobin (MetHb) is formed whe the iron component has been oxidised from the Fe^{2+} to the Fe^{3+} state, showinga characteristic brownish colour to blood unable to transport oxygen[1].

* For correspondence.

Infants present more underdeveloped and sensitive gastrointestinal tracts which are more susceptible to convert nitrate to nitrite. The nitrite circulating in the body produces methemoglobin. Although MetHb is oxygen-rich, it does not release oxygen into the bloodstream[2].

Nitrate poisoning in infants represents one of the most encountered toxic pathologies reported more frequently in many regions due to nitrate-contaminatedwell water[3]. Although governmental measures have been taken in accordance with the applicable drinking water quality law, and despite information and education of the population related to the importance of the safe water supply, this pathol- ogy is still present, especially in rural areas[4,5]. The normal blood MetHb level is between 1 and 3%. A low oxygen delivery is clinically manifested when the MetHb concentration reaches 10% or higher. The main symptom is cyanosis, and at a levelof MetHb higher than 80%, asphyxia and death may occur[4].

Children under five years of age are much more susceptible to many environmental factors than older children and adults[6], except pregnant women and peoplewith glucose-6-phosphate dehydrogenase enzyme deficiency or methemoglobin reductase.

We report a series of 23 infants diagnosed with methemoglobinemia attribut-able to ingestion of water and vegetables attended at the pediatric department overa period of 5 years.

EXPERIMENTAL

A retrospective study was conducted on 23 infants diagnosed with methemo- globinemia over a period of 5 years, between 1st January 2014 and 1st January 2019. The patients were evaluated at the Pediatric Department of Clinical County Emergency Hospital of Constanta from Romania with the age between 0–8 months(i.e. 19 infants were under 3 months old, 3 infants between 3–6 months old and 1case at 8-month-old). From the total of patients, 12 were girls and 11 were boys. Underlying pathologies that could lead to increased values of methemoglobinemia were excluded. Clinical examination (including the presence of a blood specimenthat remain chocolate brown colour), medical history, symptoms, gender, weight and food consumption were collected. Gasometry (by measuring partial pressureof O_2 (pO_2) with pO_2 = 75–100 mmHg as normal values, pCO_2 with pCO_2 = 35–45mmHg as normal values, pH with 7.35–7.45 as normal values, MetHb with 1–2%as normal values) and nitrituria using a reagent strip were collected. Agreement ofClinical County Emergency Hospital of Constanta from Romania and informed consent of the infants parents were obtained.

RESULTS AND DISCUSSION

At admission, from 12 girls and 11 boys, tachycardia between 135 and 180 beats/min was present only at the patients who presented MetHb between 61 and 70%.The weight of the infants varied between 8550 and 9900 g and all were from rural area.

The lack of running water in rural areas increases the intake of ground or surface water, which could be easily contaminated by animals, fertiliser or waste. On the day of admission, infants under 3 months were fed with milk formula (82.62%), those between 3–6 months received tea and milk formula (13.04%) and those at more than 6 months received puree of mixed vegetables (4.34%). The symptoms consisted of cyanosis, irritability, diarrhea, vomiting and tachypnea forall the infants and tachycardia was seen only at the infants ages less than 3 monthsand more than 6 months. Moreover, pO_2 seems to have slightly increased values(i.e. 103 for infants less than 3 months, 105 for infants between 3–6 months and 108 mm Hg for infants with more than 6 months). Regarding pCO_2, we found lowervalues (i.e. 28 for infants less than 3 months, 30 for infants between 3–6 monthsand 32 mm Hg for infants with more than 6 months). Normal pH and positive nitrituria were noticed in all infants (Table 1).

Table 1. Characteristics of infants

Characteristics	< 3 months age (N = 19)	3–6 month age (N = 3)	>6 month age (N = 1)
Gender	10 girls 9 boys	2 girls 1 boy	1 boy
Weight (g)	8550–9100	8700–9800	9600–9900
Food consumption	milk formula	tea and milk formula	puree of mixed vegetables
Symptoms	cyanosis, irritability, diarrhea, vomiting, tachycardia, tachypnea	cyanosis, irritability diarrhea, vomiting, tachypnea	cyanosis, irritability, diarrhea, vomiting, tachycardia, tachypnea
Gasometry			
pO_2*	103	105	108
pCO_2**	28	30	32
pH***	7.35	7.38	7.40
Nitrituria	positive	positive	positive

*Normal values = 75–100 mm Hg; **Normal values = 35–45 mm Hg ; ***Normal values = 7.35–7.45.

MetHb varied between 10–70% (Fig. 1). Therefore, 52.17% had MetHb between 10–31%, 34.78% had MetHb between 31–60%, and 13.04% had MetHb between 61–70%. About 12 cases (11 under 3 months and a 5-month-old infant)had a MetHb concentration between 10–30%. They were admitted for irritability, cyanosis of the lips, irritability associated with diarrhea and vomiting.

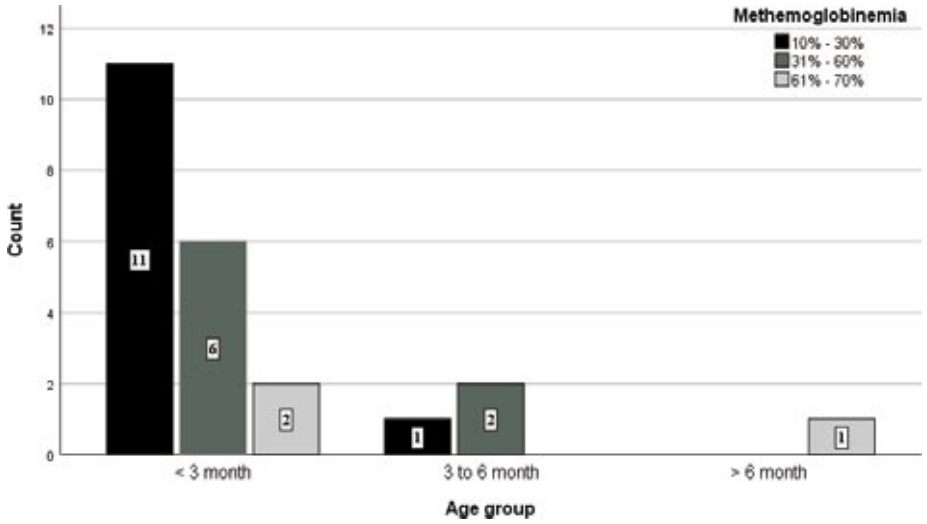


Fig. 1. Distribution according to age group and MetHb value

Eight cases presented MetHb values between 31–60% (6 under 3 months, and 2 between 3 and 6 months). The clinical presentation was severe, with irritabilityand inconstant agitation periods, cyanosis, tachypnea, and diarrhea. The soil wastreated regularly with fertiliser.

Three cases presented alarming higher MetHb levels 61–70%, 2 small infantsunder 3 months and 1 at more than 6-month old. They presented generalised cya- nosis, irritability, diarrhea, vomiting, tachypnea and tachycardia, Glasgow Coma Scale between 8–11 points. The 8-month old presented a more severe evolution.After 2 days since admission, they presented the first hemolysis signs, with pro- gressively increasing jaundice, 7 points on the Glasgow Coma Scale.

Clinical manifestations related to methemoglobinemia have been frequentlyreported in infants. Being a common cause, it has been associated with different conditions such as diarrhea, in which alimentary methemoglobinuria seems the most important cause[7,8]. Consumption of high-nitrate water, usually from mixed infant formula[9] or from vegetables, including food-borne nitrates used as food preservatives seems to have a negative impact regarding this intoxication and alsofor other diseases[10–13].

In our study, considering the fact that where no other additional episode of cyanosis, the hereditary or methemoglobinemia caused by exposure to drugs (i.e.hemoglobinopathies) were excluded.

In the last decade, more infants younger than 6 months have presented a high prevalence at nitrate-induced methemoglobinemia, based on the low gastric pH, nitrate-induced bacteria and the rudimentary of reduced nicotinamide dinucleotide-reductase system or more susceptible to other diseases[14,15]. This could be due to different soil characteristics when the nitrogenous fertilisers are frequently used[16]. However, it is very important to note the vegetables with high nitrate concentration from the respective region in order to property counsel the infant feeding[17]. The compositions of vegetables are most of the time associated with increased temperature[16]. Interestingly, nitrituria determined in all infants could be further detected in the urine, considering the extrahepatic metabolic pathway[18].

In our study, treatment consisted of intravenous vitamin C rather than meth-ylene blue in all cases. All patients recovered promptly and were discharged fromthe hospital between 12 and 24 h after admission. The treatment response was favourable from the first dose. In 4 cases a second administration was necessary,with a favourable outcome. The association between diarrhea in infants and nitrate poisoning noticed in previous studies in our area suggests that contaminated ground-water continues to be an important infant public health problem in our area[6,19]. Age at risk is not limited at the first 6 months of life and detection of gasometry and nitrituria in a cyanotic infant may sustain the diagnosis of methemoglobinemia.

CONCLUSIONS

Our study showed that the infants are the most exposed to nitrate poisoning. Although the outcome in all the cases was favourable, the long-term effects with negative impact could be severe. Clinical diagnosis and treatment for methemoglobinemia are not enough. Preventive strategies and health care programs are needed especially in rural areas in order to identify and eliminate the sources of nitrate exposure.

REFERENCES

1. M. D. COLEMAN, N. A. COLEMAN: Drug-induced Methaemoglobinaemia: Treatment Issues. Drug Saf, **14**, 394 (1996).
2. R. O. WRIGHT, W. J. LEWANDER, A. D. WOOLF: Methemoglobinemia: Etiology, Pharmacol-ogy, and Clinical Management. Ann Emerg Med, **34** (5), 646 (1993).
3. L. KNOBELOCH, B. SALNA, A. HOGAN, J. POSTLE, H. ANDERSON: Blue Babies and Nitrate-contaminated Well Water. Environ Health, **108** (7), 675 (2000).
4. H. J. M van GRINSEVEN, M. H. WARD, N. BENJAMIN, T. M. de KOK: Does the Evidence about Health Risks Associated with Nitrate Ingestion Warrant an Increase of the Nitrate Standard for Drinking Water? Environ Healt, **5**, 26 (2006).
5. C. M. MIHAI, M. R. STOICESCU, A. BALASA, L. MIHAI: *E. coli* Infection and Nitrate Poisoning from Drinking Water in Infants from

Constanta County. Pediatric Research, **68**, 436(2010).
6. S. C. CAMBREA, L. C. PETCU, C. M. MIHAI, T. L. HANGAN, D. M. ILIESCU: Influence of Environmental Factors about Evolution of Shigellosis in Constanta County of Romania. J Environ Prot Ecol, **20** (2), 986 (2019).
7. D. PHILLIPS, R. GRADISEK, D.E. HEISELMAN: Methemoglobinemia Secondary to AnilineExposure. Emerg Med, **1**, 425 (1990).
8. H. KRAVITZ, L. D. ELEGANT, K. E. KAISER: Methemoglobin Values in Premature and Mature Infants and Children. Am J Dis Child, **91**, 1 (1956).
9. J. SANCHEZ-ECHANIZ, J. BENITO-FERNANDEZ, S. MINTEGUI-RASO: Methemoglobine mia and Consumption of Vegetables in Infants. Pediatrics, **107**, 1024 (2001).
10. S. DIACONU, S. C. CAMBREA, L. C. PETCU, S. RUGINĂ: Aspects of Nosocomial Gastro-enteritis with Rotavirus in Children Hospitalized in Constanta – Romania. Acta Medica Mediter-ranea, **33** (Special Issue 2), 1289 (2017).
11. T. Y. CHAN: Food-borne Nitrates and Nitrites as a Cause of Methemoglobinemia. Southeast Asian J Trop Med Public Health, **27**, 189 (1996).
12. C. ZUGRAVU, M. I. NANU, F. MOLDOVANU et al.: The Influence of Perinatal Education on Breastfeeding Decision and Duration. International Journal of Child Health and Nutrition, **7** (2), 74 (2018).
13. S. BERBECE, D. ILIESCU, V. ARDELEANU, A. NICOLAU, R.C. JECAN: Use of Phos- phatidylcholine in the Treatment of Localized Fat Deposits Results and expectations. Revista de Chimie, **68** (7), 1438 (2017).
14. E. S. POLLACK, C. V. POLACK, Jr.: Incidence of Subclinical Methemoglobinemia in Infants with Diarrhea. Ann Emerg Med, **24**, 652 (1994).
15. S. C. CAMBREA, M. M. ILIE, E. DUMEA et al.: Correlation of an Acute Viral Hepatitis TypeA Outbreak in the Constanta County with Environmental Risk Factors. J Environ Prot Ecol, **15**(1), 340 (2014).
16. American Academy of Pediatrics, Committee on Nutrition. Infant Methemoglobinemia: the Role of Dietary Nitrate. Pediatrics, **46**, 475 (1970).
17. O. UYGUR, S. ESEN, S. TANRIVERDE et al.: Noninvasive Diagnosis and Continuous Moni- toring of Local Anesthetic-induced Methemoglobinemia in a Neonate. Indian J Hematol BloodTransfus, **30** (Suppl 1), 180 (2014)
18. K. F. MURRAY, D. L. CHRISTIE: Dietary Protein Intolerance in Infants with Transient Methe- moglobinemia and Diarrhea. J Pediatr, **122**, 90 (1993).
19. F. R. GREER, M. SHANNON: Infant Methemoglobinemia: the Role of Dietary Nitrate in Food and Water. Pediatrics, **116** (3), 784 (2005).

Received 10 October 2019
Revised 12 November 2019

Neurological manifestations found in children with multisystem inflammatory syndrome

CRISTINA MARIA MIHAI[1], TATIANA CHISNOIU[1], CLAUDIA SIMONA CAMBREA[2], CORINA ELENA FRECUS[1], LARISIA MIHAI[1], ADRIANA LUMINITA BALASA[1], ALINA ZORINA STROE[3], ANCA ELENA GOGU[4] and ANY DOCU AXELERAD[3]

Departments of [1]Pediatrics, [2]Infectious Diseases and [3]Neurology, Faculty of General Medicine, 'Ovidius' University of Constanta, 900470 Constanta; [4]Department of Neurology, 'Victor Babes' University of Medicine and Pharmacy, 300041 Timisoara, Romania

Received October 18, 2021; Accepted November 17, 2021

DOI: 10.3892/etm.2022.11187

Abstract. The pandemic that resulted from the spread of SARS-CoV-2 viral infections has affected the population worldwide but has characteristically shown a preponderance for affecting adults. However, cases of SARS-CoV-2 infection have been reported in children, showing a systemic echo and severe damage. Multisystem inflammatory syndrome in children (MIS-C) can occur, on average, 4 weeks after the infection of a child with SARS-CoV-2. The aim of the present study was to examine 30 cases of children affected by MIS-C in terms of symptoms, laboratory tests, and evolution. Patients included in the study presented with neurological symptomatology including headache, meningism, and drowsiness. Treatment was administered in concordance with the protocol for MIS-C. The evolution of the patients in the present study was favorable and the symptomatology remitted in days to weeks. The importance of identifying the features of this disease, its treatment, and that the most probable evolution is favorable is significant in the medical world, especially as the pandemic is ongoing.

Introduction

A worldwide pandemic was triggered with the manifestation of the SARS-CoV-2 virus (COVID-19). Millions of individuals have since succumbed and millions more have been infected (1). Fever, cough, shortness of breath, tiredness, and malaise are the primary clinical symptoms of this disease in adults (2). Although infections in children were considerably fewer than in adults at the start of the epidemic, with the introduction of quarantine and spending time only indoors, more cases began to appear in children who came into close contact with infected family members (3).

Despite the rise of the COVID-19 pandemic in Asia and its rapid spread across the globe, doctors were relieved that the virus affected the majority of children with minor symptoms (4). However, in April 2020, the United Kingdom's National Health Service issued a warning regarding cases of school-aged children and adolescents with fever, hypotension, severe abdominal pain, and cardiac dysfunction who screened positive for SARS-CoV-2 infection via a nasopharyngeal RT-PCR or antibody (Ab) test (4). Due to their elevated levels of interleukin (IL)-6, these patients often needed inotropic support to improve cardiac output, with just a few needing extracorporeal membrane ventilation. After a few days, the majority of the children were no longer in need of critical care and had completely recovered. However, a few children succumbed as a result of complications associated with extracorporeal membrane oxygenation (3,4).

Young patients with acute COVID-19 infection may be asymptomatic or they may have a fever, cough, nasal congestion, and/or digestive symptoms (4), and often do not require hospitalization. However, in some cases, children may develop a multisystemic inflammatory syndrome after becoming infected with the SARS-CoV-2 virus, a condition that has been termed multisystem inflammatory syndrome in children (MIS-C). Throughout the UK (5), Italy (6), Spain (7), France (8), Switzerland (8), and the US (8), the Centers for Disease Control and Prevention developed a case definition for use in the US and termed it MIS-C.

During the SARS-CoV-2 virus pandemic, pediatricians observed fever and multisystem inflammation as being present in hospitalized children. In several cases, the patients were severely affected with multiorgan shock and collapse that necessitated intensive care, and several cases presented with characteristics of Kawasaki disease (KD) or KD shock syndrome (6,8-10).

Correspondence to: Dr Alina Zorina Stroe, Department of Neurology, Faculty of General Medicine, 'Ovidius' University of Constanta, 1 Aleea Universității Street, 900470 Constanta, Romania
E-mail: zorina.stroe@yahoo.com

Abbreviations: MIS-C, multisystem inflammatory syndrome in children; PICU, pediatric intensive care unit; KD, Kawasaki disease; Ab, antibody; CT, computed tomography

Key words: multisystem inflammatory syndrome in children, SARS-COV2, coronavirus infection, pediatrics

Between 3 March and 14 April 2020, only 48 children required admission to a pediatric intensive care unit (PICU), according to an early retrospective research assessing pediatric outcomes at 46 pediatric institutions throughout North America (11). The United Kingdom's Pediatric Intensive Care Society issued a warning about an increase in the number of children presenting with a multisystem inflammatory disease resembling KD or toxic shock syndrome, with many children testing positive for COVID-19 (12). The New York State Department of Health reported 15 first cases with symptoms consistent with those described above on 4 May 2020, and by 17 May 2020, 145 suspected cases had been recorded in New York state hospitals (11).

MIS-C has been detected in a number of nations affected by the COVID-19 pandemic. Starting from the spring of 2020, the above disease has been referred to as a Kawasaki-like disease in Western nations (5). MIS-C is a hyperinflammatory condition that can progress to macrophage activation syndrome, or cytokine storm, as reported by Kabeerdoss *et al* (13). Neurological manifestations that correlated with SARS-CoV-2 infection present in adults included encephalitis, meningitis, encephalopathy, stroke, seizures, and anosmia (14). However, there were limited instances of neurological symptoms associated with SARS-CoV-2 infection in children (15).

The aim of the present study was to examine 30 cases of children affected by MIS-C in terms of symptoms, laboratory tests, and evolution. The evolution of the disease was favorable, which is encouraging for the medical field.

Patients and methods

Ethics and consent. A retrospective, observational, descriptive study was conducted between August 2020 and May 2021 in the Pediatrics Clinic of Constanta County Emergency Hospital. The present study was authorized by the Ethics Committee of the Constanta Clinical Hospital in Romania (no. 24/22.07.2020), and all the guardians completed an informed consent form. All measures to ensure the safety of hospital staff were adhered to.

Inclusion and exclusion criteria. The inclusion criteria for the study were patients with signs, symptoms, and elevated laboratory markers who met the CDC case definition criteria for MIS-C, and who presented with multisystemic and neurological impairment. The exclusion criterion was the existence of a pre-existing neurological condition.

Patient data. The study included 30 patients of age ranging for both boys and girls between 8 months and 15 years. The laboratory data, imaging studies including a craniocerebral MRI, and confirmation of SARS-CoV-2 infection via igG anti-SARS-CoV-2 antibodies (method: microparticle chemiluminescence, CMIA), therapy, and evolution were collected for each patient.

Results

General. In the present study, 30 patients with MIS-C were identified, of whom 14 had neurological manifestations. Of these 14, 10 patients (71%) were male.

Table I. Symptoms of the study group with MIS-C.

Symptoms	No. of patients
Headache	11
Ataxia	5
Photophobia	5
Difficulty walking	4
Meningism	9
Diplopia	1
New appeared strabismus	1
Drowsiness	7
Lethargy	5
Alteration of consciousness	6
Skin hyperaesthesia	6

MIS-C, multisystem inflammatory syndrome in children.

Symptoms experienced by MIS-C patients. The most common neurological manifestations in MIS-C were headache (11 cases), meningism (9 cases), and drowsiness (7 cases). Skin hyperesthesia and altered consciousness were reported in 6 patients. Patients experienced photophobia, ataxia, and lethargy in 5 cases, respectively. One patient from each of the studied groups presented new diplopia and strabismus, and subsequently a craniocerebral MRI was performed, from which demyelinating lesions were detected. The symptomatology is described in Table I.

Clinical features. According to the treatment protocol from MIS-C, all patients in the study group received human immunoglobulin IV at a dose of 2 g/kg for a single administration and acetylsalicylic acid at a dose between 3 and 5 mg/kg/day. In addition, methylprednisolone was administered to 5 patients at a dose of 3 mg/kg/day and 7 patients at a dose of 5 mg/kg/day. The detailed information of some cases is provided in Table II. No patient required inotropic medication.

Discussion

MIS-C. MIS-C following SARS-CoV-2 infection is characterized by persistent fever, increased inflammatory markers, and multisystemic impairment (16). MIS-C can occur, on average, 4 weeks after infection of a child with SARS-CoV-2. The child may have acquired the virus via an asymptomatic contact, and in certain instances, the youngster and their family may be unaware they have been infected. Multisystem damage most often includes heart, mucocutaneous, gastrointestinal, and neurological damage.

The report by Abdel-Mannan *et al* in April 2020 described 52% of neurological symptoms associated with MIS-C, including headache, lethargy, altered consciousness, encephalopathy, dysarthria/dysphonia, hallucinations, ataxia, and convulsions (17).

Neurological manifestations in MIS-C are common and often include headache, lethargy, confusion, and irri- tability. More severe neurological manifestations, such as

Table II. Patient demographics and clinical features.

No.	Sex	Age	Neurological manifestations	Neurological investigations	Proof of infection for SARS-CoV-2	Inflammatory markers: PCR (mg/dl) ESR (mm/h) Fibrinogen (mg/dl) NT-proBNP (pg/ml) IL-6 (pg/ml)	Treatment	Evolution
1.	F	15 years 10 months	Difficulty walking; Headache; Meningism; Photophobia; Drowsiness; Lethargy; Alteration of consciousness	Craniocerebral CT-normal	SARS-CoV-2 Ab IgG -2.98	PCR (13.86) ESR (29) Fibrinogen (410) Feritin (139) NT-proBNP (2,320) D Dimer (4.54)	-IG IV (2 g/kg) -Acetylsalicylic acid	Complete recovery without neurological sequelae
2.	F	2 years 4 months	Photophobia; Lethargy; Drowsiness; Difficulty walking	None	SARS-CoV-2 Ab IgG -4.87	PCR (2.8) ESR (52) Fibrinogen (594) Feritin (339) NT-proBNP (214) D Dimer (2.32)	-IG IV (2 g/kg) -Acetylsalicylic acid	Complete recovery without neurological sequelae
3.	M		Meningism; Headache; Skin hyperaesthesia; Difficulty walking	Craniocerebral CT-normal	SARS-CoV-2 Ab IgG -6.26	PCR (9) ESR (16) Fibrinogen (476) Feritin (357) NT-proBNP (1,010) D Dimer (2.9)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (3 mg/kg/day)	Complete recovery without neurological sequelae
4.	M		Headache; Alteration of consciousness; Drowsiness	EEG-normal	SARS-CoV-2 Ab IgG -5.07	PCR (5.53) ESR (37) Fibrinogen (360) Feritin (539) NT-proBNP (586) D Dimer (2.34)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (3 mg/kg/day)	Complete recovery without neurological sequelae
5.	M		Headache; Diplopia; Photophobia; Meningism; New strabismus appeared	CT MRI-demyelinating lesions	SARS-CoV-2 Ab IgG -2.02	PCR (18.31) ESR (87) Fibrinogen (762) Feritin (208.9) NT-proBNP (586) D Dimer (1.2)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (3 mg/kg/day)	Complete recovery without neurological sequelae

Table II. Continued.

No.	Sex	Age	Neurological manifestations	Neurological investigations	Proof of infection for SARS-CoV-2	Inflammatory markers: PCR (mg/dl) ESR (mm/h) Fibrinogen (mg/dl) NT-proBNP (pg/ml) IL-6 (pg/ml)	Treatment	Evolution
6.	M		Meningism; Photophobia; Diplopia; Headache; Alteration of consciousness; Skin hyperaesthesia		SARS-CoV-2 Ab IgG -10.3	PCR (27.51) ESR (87) Fibrinogen (483) Feritin (208.9) NT-proBNP (2,074) D Dimer (5.14) IL-6 (75)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (3 mg/kg/day)	Complete recovery without neurological sequelae
7.	M		Meningism; Photophobia; Lethargy; Ataxia; Skin hyperaesthesia	Normal craniocerebral CT	SARS-CoV-2 Ab IgG -7.39	PCR (11.58) ESR (43) Fibrinogen (543) Feritin (725.9) NT-proBNP (1,823) D Dimer (5.23)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (3 mg/kg/day)	Complete recovery without neurological sequelae
8.	M		Meningism; Headache; Alteration of consciousness	Normal craniocerebral CT	SARS-CoV-2 Ab IgG -14.2	PCR (15.65) ESR (73) Fibrinogen (664) Feritin (645.5) NT-proBNP (1,578) D Dimer (3.11)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae
9.	M		Headache; Irritability; Lethargy; Alteration of consciousness; Ataxia; Skin hyperaesthesia	Normal craniocerebral CT	SARS-CoV-2 Ab IgG -4.78	PCR (18.78) ESR (72) Fibrinogen (653) Feritin (391.8) NT-proBNP (1,027) D Dimer (1.73)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae
10.	M		Headache; Irritability; Lethargy;		SARS-CoV-2 Ab IgG -4.78	PCR (10.69) ESR (41) Fibrinogen (510) Feritin (291.7) NT-proBNP (858) D Dimer (2.82)	-IG IV (2 g/kg) -Acetylsalicylic acid Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae

Table II. Continued.

No.	Sex	Age	Neurological manifestations	Neurological investigations	Proof of infection for SARS-CoV-2	Inflammatory markers: PCR (mg/dl) ESR (mm/h) Fibrinogen (mg/dl) NT-proBNP (pg/ml) IL-6 (pg/ml)	Treatment	Evolution
11.	F		Headache; Drowsiness; Alteration of consciousness; Meningism; Ataxia; Skin hyperaesthesia		SARS-CoV-2 Ab IgG -7.01	PCR (30.68) ESR (120) Fibrinogen (607) Feritin (677.7) NT-proBNP (6,559) D Dimer (4.69)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae
12.	M		Meningism; Drowsiness; Skin hyperaesthesia; Headache		SARS-CoV-2 Ab IgG -7.01	PCR (12.54) ESR (54) Fibrinogen (569) Feritin (500.6) NT-proBNP (2,455) D Dimer (2.09)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae
13.	M		Headache; Ataxia; Drowsiness; Meningism		SARS-CoV-2 Ab IgG -6.5	PCR (12.2) ESR (30) Fibrinogen (520) Ferritin (250) NT-proBNP (500) D Dimeri (1.7)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae
14.	F		Drowsiness; Lethargy; Skin hyperaesthesia; Ataxia; Difficulty walking		SARS-CoV-2 Ab IgG -12.2	PCR (9.1) ESR (25) Fibrinogen (610) Feritin (145) NT-proBNP (956) D Dimer (1.17)	-IG IV (2 g/kg) -Acetylsalicylic acid -Methylprednisolone (5 mg/kg/day)	Complete recovery without neurological sequelae

M, male; F, Female; CT, computed tomography; PCR, polymerase chain reaction; ESR, erythrocyte sedimentation rate; NT-proBNP, natriuretic peptide.

encephalopathy, seizures, coma, stroke, meningoencephalitis, and meningitis, occur at a much smaller percentage, as recently reported (17,18).

A study conducted in the US showed that of 616 patients with MIS-C, 20% had neurological manifestations (19). In addition, severe, life-threatening neurological disorders occurred in 20 patients (3%), including severe encephalopathy, demyelinating lesions, stroke, acute cerebral edema, and Guillain-Barre syndrome.

In the present study, treatment was provided according to the current recommendations of the international treatment guidelines for MIS-C with immunoglobulin, methylprednisolone, and acetylsalicylic acid. The evolution was favorable in all cases, with recovery without neurological sequelae. In adults, human plasmapheresis or intravenous immunoglobulin was reported to be beneficial for neuroinflammatory complications, including COVID-19-linked autoimmune meningoencephalitis (20). In children, the therapeutic effect of plasmapheresis remains unclear. All the patients with MIS-C who received intravenous immunoglobulin and/or methylprednisolone fully recovered without neurological sequelae (21).

Cheung *et al* (22) included 17 patients in their study, all of whom presented fever, 14 presented gastrointestinal symptoms of whom the majority presented mucocutaneous findings, and 13 patients experienced shock. All 17 patients had increased levels of inflammatory markers, the majority having lymphopenia, and all were positive for SARS-CoV-2 infection. There were no deaths among the patients and they were all released (21).

Until the end of April 2020, SARS-CoV-2 infection in children was believed to be asymptomatic or to produce moderate febrile sickness (23). Riollano-Cruz *et al* (23) described 15 pediatric COVID-19 patients that manifested symptoms consistent with a multisystemic hyperinflammatory syndrome that overlapped several characteristics with well-characterized systemic inflammatory diseases. Not all patients tested positive for SARS-CoV-2 through molecular testing of nasopharyngeal specimens in their research. However, all the patients tested positive for SARS-CoV-2 in lower respiratory specimen test, indicating that, although COVID-19 infection produced this symptomatology, the hyperinflammatory condition observed in these children was probably triggered by a post-infectious cytokine storm instead of direct viral replication-induced cell damage.

The relative absence of pulmonary findings in pediatric COVID-19 cases compared to adults raises further concerns regarding pathogenesis, since pulmonary involvement does not appear to be the main source of dysfunction in SARS-CoV-2 infection in children (24). Brown *et al* (24) reported infiltration by $CD45RO^+$ T lymphocytes (activated or memory) and cytotoxic $CD8^+$ T lymphocytes in coronary artery aneurysms from patients with fatal acute coronary arteritis KD, concluding that this finding suggests the existence of an intraepithelial pathogen, most probably viral, continuing to support an endothelium-mediated cause. Varga *et al* (25) recently reported the presence of virus-like particles in endothelial cells and endotheliitis in COVID-19 patient tissues. Endothelial cell dysfunction caused by direct viral infection may account for the poor systemic microcirculatory function observed in COVID-19 patients.

There have been documented differences in disease outcomes across races and ethnic groups following SARS- CoV-2 infection, with individuals of African descent (21), African Americans (22), Hispanics (23), and individuals from Latin America (24) experiencing more severe sickness, hospitalizations, and fatalities. The lineage of COVID-associated MIS-C patients demonstrated the essential nature of preventing viral transmission in this age range.

Differences between the KD and MIS-C. Doctors have identified clinical parallels between MIS-C and KD, a febrile disease that occurs in early infancy and leads to inflammation of the blood vessels, which may result in coronary artery aneurysms (25). Patients with MIS-C may exhibit comparable symptoms to those with KD, including fever, dilated conjunctival vessels, rash, and oropharyngeal erythema (25,26). Nevertheless, these clinical signs are not diagnosis-specific and may be identified in a variety of infectious diseases in children.

In the last 50 years, the epidemiology of KD has been documented globally, with 80 percent of cases occurring in children under the age of five years and a peak incidence at 10 months of age. This is in striking contrast to the epidemiology of MIS-C, a disease that mostly affects older children and adolescents. Laboratory data consistent with MIS-C, such as leukopenia and extremely high ventricular natriuretic peptide values, were not consistent with KD. Asian children have the greatest prevalence of KD worldwide, while African-born children are more susceptible to MIS-C. In China or Japan, no instances of MIS-C have been reported (26). Both diseases have very distinct epidemiologies. While SARS-CoV-2 has not been definitively linked to MIS-C, the observation that MIS-C occurred during COVID-19 outbreaks in Europe and the US supports this theory.

Link between MIS-C and SARS-CoV-2. Whether or not MIS-C is associated with SARS-CoV-2 infection, the pathophysiological mechanism of the disease remains unclear. Other authors have suggested that, MIS-C is not the consequence of acute viral infection, but rather a post-infectious phenomenon associated with exacerbation of the disease mediated by IgG antibodies (25,26). This phenomenon occurs for two main reasons. First, MIS-C cases have been delayed in certain regions relative to the peak of SARS-CoV-2 infection. Due to the likelihood that children may become infected with the virus owing to exposure to infected parents as a result of home limitations, the maximum number of cases is extended. Interestingly, children with MIS-C often appear with gastrointestinal symptoms and have minimal, if any, respiratory symptoms. As a result, the virus may spread mostly via the gastrointestinal system. Enterocytes have been found to be susceptible to infection by SARS-CoV-2, and patients with MIS-C who have had exploratory laparotomy exhibit mesenteric adenitis, which facilitates gastrointestinal infection. This has not been observed in children suffering with MIS-C (27). Furthermore, the development of antibodies against SARS-CoV-2 does not always suggest a post-infectious phase, since antibodies may develop as early as the second week after infection.

There is a dearth of information on the specificity of Ab tests conducted on individuals with MIS-C, which may vary significantly. As SARS-CoV-2 infection spreads across

a community and most children remain asymptomatic or slightly symptomatic, positive Ab tests may be more prevalent, and child screening may be needed to demonstrate a positive link between SARS-CoV-2 and a specific disease. It is intriguing that worsening of the disease in patients with COVID-19 treated with convalescent plasma is not yet recognized as a clinical issue, as one would anticipate if Ab-mediated enhancement represents a significant mechanism for the occurrence of severe COVID-19 complications. Kaushik *et al* (28) conducted a meta-analysis of 328 publications and found that, the median period between the start of symptoms and hospital admission was four days (interquartile range, 3-6 days). The most often reported symptom was fever, being followed by gastrointestinal (GI) symptoms. Almost all investigations documented gastrointestinal signs, which were observed in 458 (70%) individuals, with symptoms resembling viral gastroenteritis or inflammatory bowel disease, including nausea, vomiting, diarrhea, and abdominal discomfort. At presentation, 332 (51%) individuals reported having cardiovascular symptoms. An additional 186 (28%) individuals experienced hypotension, and 235 (36%) patients experienced ER-like symptoms, with 41 having a conventional KD and 194 having an unusual presentation. Among patients exhibiting KD-like symptoms, 62 (26%) developed circulatory shock (29-31). In 12 studies, the central nervous system was involved in the evolution of 145 (22%) children who presented with aseptic meningitis, headache, or changed mental state (32). Approximately 8 studies documented symptoms of the respiratory system, such as coughing or constipation (33). Rash was observed in 379 (58%) patients, hand and foot edema was mentioned in 83 (13%) patients, conjunctival injection was noted in 263 (40%) patients, cracked red lips were noted in 148 (23%) patients, strawberry tongue was revealed in 29 (4.5%) patients, and cervical lymphadenitis was identified in 27 (4%) patients. In terms of presenting features reported across geographic areas, rashes were more often observed in India, whereas cardiovascular involvement was more frequently recorded in European research.

A convincing alternate theory for the prominent cytokine storm in children with MIS-C is centered on the known inhibitory activity on type I and III interferon responses of coronaviruses (34), which may also result in a delayed cytokine storm in patients with an immune system incapable of controlling the viral response or replication, or in patients with elevated initial SARS-CoV-2 viral load (35).

Panigrahy *et al* (36) reviewed 57 studies of 875 MIS-C patients from 15 countries, and the patients had tested positive or had an epidemiologic connection with SARS-CoV-2 infection. More boys than girls presented with symptoms, and the age of 9 years was the most prevalent in the children in this review. Almost 50% of the patients presented with other medical conditions and the most prevalent was obesity. In that study, the death rate related to MIS-C was 2.5% (36).

In summary, MIS-C is a relatively novel and understudied disease that affects children and is therefore of significant interest globally. To the best of our knowledge, this is the first study of MIS-C in Romania; consequently, the insights provided by this study are extremely valuable and significant. The evolution of the patients in the present study was favorable and the symptomatology remitted in days to weeks.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CMM, TC, CSC, CEF, LM, ALB, AZS, AEG and ADA conceived and designed the study. CMM, TC, CSC, CEF and AZS performed the literature research. CMM, TC, CSC, CEF, LM, ALB, AZS and ADA were involved in the interpretation of the results. CMM, TC, CSC, CEF, LM, ALB, AZS and ADA were involved in the writing of the manuscript. All authors have read and approved the final manuscript. CMM, TC, CSC, CEF, LM, ALB, AZS, AEG and ADA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of the Constanta Clinical Hospital, Romania (no. 24/22.07.2020) and all the guardians provided informed consent and signed a statement to that effect.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bialek S, Boundy E, Bowen V, Chow N, Cohn A, Dowling N, Ellington S, Gierke R, Hall A, MacNeil J, *et al*: Severe outcomes among patients with coronavirus disease 2019 (COVID-19)-United States, February 12-March 16, 2020. MMWR Morb Mortal Wkly Rep 69: 343-346, 2020.
2. Hasani H, Mardi S, Shakerian S, Taherzadeh-Ghahfarokhi N and Mardi P: The novel coronavirus disease (COVID-19): A PRISMA systematic review and meta-analysis of clinical and paraclinical characteristics. Biomed Res Int 2020: 3149020, 2020.
3. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z and Tong S: Epidemiology of COVID-19 among children in China. Pediatrics 145: e20200702, 2020.
4. Cruz AT and Zeichner SL: COVID-19 in children: Initial characterization of the pediatric disease. Pediatrics 145: e20200834, 2020.
5. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N and Theocharis P: Hyperinflammatory shock in children during COVID-19 pandemic. Lancet 395: 1607-1608, 2020.
6. Verdoni L, Mazza A, Gervasoni A, Martelli L, Ruggeri M, Ciuffreda M, Bonanomi E and D'Antiga L: An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet 395: 1771-1778,

2020.

7. Cabrero-Hernández M, García-Salido A, Leoz-Gordillo I, Alonso-Cadenas JA, Gochi-Valdovinos A, González Brabin A, De Lama Caro-Patón G, Nieto-Moro M, de-Azagra-Garde AM and Serrano-González A: Severe SARS-CoV-2 infection in children with suspected acute abdomen: A case series from a tertiary hospital in Spain. Pediatr Infect Dis J 39: e195-e198, 2020.
8. Belhadjer Z, Méot M, Bajolle F, Khraiche D, Legendre A, Abakka S, Auriau J, Grimaud M, Oualha M, Beghetti M, *et al*: Acute heart failure in multisystem inflammatory syndrome in children in the context of global SARS-CoV-2 pandemic. Circulation 142: 429-436, 2020.
9. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, Debray A, Basmaci R, Salvador E, Biscardi S, *et al*: Outbreak of Kawasaki disease in children during COVID-19 pandemic: A prospective observational study in Paris, France. MedRxiv: May 14, 2020 (Epub ahead of print). doi: https://doi. org/10.1101/2020.05.10.20097394.
10. Viner RM and Whittaker E: Kawasaki-like disease: Emerging complication during the COVID-19 pandemic. Lancet 395: 1741-1743, 2020.
11. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, Heidemann SM, Kleinman LC, Sen AI, Hall MW, *et al*: Characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. JAMA Pediatr 174: 868-873, 2020.
12. Flood J, Shingleton J, Bennett E, Walker B, Amin-Chowdhury Z, Oligbu G, Avis J, Lynn RM, Davis P, Bharucha T, *et al*: Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS): Prospective, national surveillance, United Kingdom and Ireland, 2020. Lancet Reg Health Eur 3: 100075, 2021.
13. Kabeerdoss J, Pilania RK, Karkhele R, Kumar TS, Danda D and Singh S: Severe COVID-19, multisystem inflammatory syndrome in children, and Kawasaki disease: Immunological mechanisms, clinical manifestations and management. Rheumatol Int 41: 19-32, 2021.
14. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, *et al*: Neurologic manifestations of hospital- ized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 77: 683-690, 2020.
15. Dugue R, Cay-Martínez KC, Thakur KT, Garcia JA, Chauhan LV, Williams SH, Briese T, Jain K, Foca M, McBrian DK, *et al*: Neurologic manifestations in an infant with COVID-19. Neurology 94: 1100-1102, 2020.
16. CDC: Multisystem inflammatory syndrome (MIS). Centers for disease control and prevention, 2021. https://www.cdc.gov/mis/ index.html. Access date: ?
17. Abdel-Mannan O, Eyre M, Löbel U, Bamford A, Eltze C, Hameed B, Hemingway C and Hacohen Y: Neurologic and radiographic findings associated with COVID-19 infection in children. JAMA Neurol 77: 1440-1445, 2020.
18. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF, Newburger JW, Kleinman LC, Heidemann SM, Martin AA, *et al*: Multisystem inflammatory syndrome in U.S. Children and adolescents. N Engl J Med 383: 334-346, 2020.
19. LaRovere KL, Riggs BJ, Poussaint TY, Young CC, Newhams MM, Maamari M, Walker TC, Singh AR, Dapul H, Hobbs CV, *et al*: Neurologic involvement in children and adolescents hospitalized in the United States for COVID-19 or multisystem inflammatory syndrome. JAMA Neurol 78: 536-547, 2021.
20. Dogan L, Kaya D, Sarikaya T, Zengin R, Dincer A, Akinci IO and Afsar N: Plasmapheresis treatment in COVID-19-related autoimmune meningoencephalitis: Case series. Brain Behav Immun 87: 155-158, 2020.
21. Kurup S, Burgess R, Tine F, Chahroudi A and Lee DL: SARS-CoV-2 infection and racial disparities in children: Protective mechanisms and severe complications related to MIS-C. J Racial Ethn Health Disparities: Jul 13, 2021 (Epub ahead of print). doi: 10.1007/s40615-021-01092-7.
22. Cheung EW, Zachariah P, Gorelik M, Boneparth A, Kernie SG, Orange JS and Milner JD: Multisystem inflammatory syndrome related to COVID-19 in previously healthy children and adoles- cents in New York City. JAMA 324: 294-296, 2020.
23. Ashktorab H, Pizuomo A, González NAF, Villagrana EDC, Herrera-Solís ME, Cardenas G, Zavala-Alvarez D, Oskrochi G, Awoyemi E, Adeleye F, *et al*: A comprehensive analysis of COVID-19 impact in Latin America. Res Sq: rs.3.rs-141245, 2021.
24. Brown TJ, Crawford SE, Cornwall ML, Garcia F, Shulman ST and Rowley AHCD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. J Infect Dis 184: 940-943, 2001.
25. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR,

Schuepbach RA, Ruschitzka F and Moch H: Endothelial cell infection and endotheliitis in COVID-19. Lancet 395: 1417-1418, 2020.
26. Xu S, Chen M and Weng J: COVID-19 and Kawasaki disease in children. Pharmacol Res 159: 104951, 2020.
27. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, *et al*: SARS-CoV-2 productively infects human gut enterocytes. Science 369: 50-54, 2020.
28. Kaushik A, Gupta S, Sood M, Sharma S and Verma S: A system- atic review of multisystem inflammatory syndrome in children associated with SARS-CoV-2 infection. Pediatr Infect Dis J 39: e340-e346, 2020.
29. Balasubramanian S, Nagendran TM, Ramachandran B and Ramanan AV: Hyper-inflammatory syndrome in a child with COVID-19 treated successfully with intravenous immunoglob- ulin and tocilizumab. Indian Pediatr 57: 681-683, 2020.
30. Rauf A, Vijayan A, John ST, Krishnan R and Latheef A: Multisystem inflammatory syndrome with features of atypical kawasaki disease during COVID-19 pandemic. Indian J Pediatr 87: 745-747, 2020.
31. Acharyya BC, Acharyya S and Das D: Novel coronavirus mimicking kawasaki disease in an infant. Indian Pediatr 57: 753-754, 2020.
32. Lin JE, Asfour A, Sewell TB, Hooe B, Pryce P, Earley C, Shen MY, Kerner-Rossi M, Thakur KT, Vargas WS, *et al*: Neurological issues in children with COVID-19. Neurosci Lett 743: 135567, 2021.
33. Dufort EM, Koumans EH, Chow EJ, Rosenthal EM, Muse A, Rowlands J, Barranco MA, Maxted AM, Rosenberg ES, Easton D, *et al*: Multisystem inflammatory syndrome in children in New York State. N Engl J Med 383: 347-358, 2020.
34. Park A and Iwasaki A: Type I and III interferons-induction, signaling, evasion, and application to combat COVID-19. Cell Host Microbe 27: 870-878, 2020.
35. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, Jordan TX, Oishi K, Panis M, Sachs D, *et al*: Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 181: 1036-1045.e9, 2020.
36. Panigrahy N, Policarpio J and Ramanathan R: Multisystem inflammatory syndrome in children and SARS-CoV-2: A scoping review. J Pediatr Rehabil Med 13: 301-316, 2020.

Diagnostics

MDPI

Article

Impact of SARS-CoV-2 Infection in Patients with Neurological Pathology

Any Docu Axelerad [1,†], Lavinia Florenta Muja [1,†], Alina Zorina Stroe [1,*], Lavinia Alexandra Zlotea [2], Carmen Adella Sirbu [3], Silviu Docu Axelerad [4], Dragos Catalin Jianu [5], Corina Elena Frecus [6] and Cristina Maria Mihai [6]

[1] Department of Neurology, General Medicine Faculty, Ovidius University, 900470 Constanta, Romania; axelerad.docu@365.univ-ovidius.ro (A.D.A.); lavinia.muja@365.univ-ovidius.ro (L.F.M.)
[2] Radiology-Medical Imaging, Sf. Apostol Andrei Emergency County Clinical Hospital Constanta, 900591 Constanta, Romania; laviniazlotea23@gmail.com
[3] Department of Neurology, Titu Maiorescu University, 040441 Bucharest, Romania; carmen.sirbu@prof.utm.ro
[4] Faculty of General Medicine, 'Vasile Goldis' University, 317046 Arad, Romania; docu-axelerad.silviu@student.uvvg.ro
[5] Department of Neurology, Victor Babes University of Medicine and Pharmacy, 300041 Timisoara, Romania; jianu.dragos@umft.ro
[6] Department of Pediatrics, General Medicine Faculty, Ovidius University, 900470 Constanta, Romania; corina.frecus@365.univ-ovidius.ro (C.E.F.); cristina_mihai@365.univ-ovidius.ro (C.M.M.)
* Correspondence: alina.stroe@365.univ-ovidius.ro; Tel.: +40-727987950
† These authors contributed equally to this work.

Abstract: The COVID-19 disease, caused by infection with SARS-CoV-2, rapidly transformed into a pandemic following its emergence, and it continues to affect the population at a global level. This disease is associated with high mortality rates and mainly affects the pulmonary spectrum, with signs of interstitial pneumonia or other pathological modifications. Signs indicative of SARS-CoV-2 infection can be observed using pulmonary radiography or computed tomography scans and are closely linked to acute respiratory distress; however, there is accumulating evidence that the virus affects the central nervous system. Several symptoms, such as headaches, cough, fatigue, myalgia, ageusia, and anosmia, have also been reported along with neurological syndromes such as stroke, encephalopathy, Guillain–Barre syndrome, convulsions, and coma; the most frequent associated complication is ischemic stroke. Diagnosis of infection with SARS-CoV-2 virus is based on a positive RT-PCR test. Imaging investigations, such as thoracic computed tomography scans, are not used to diagnose COVID-19, monitor for pulmonary disease, or follow dynamic disease evolution, but they may be used in the case of a negative RT-PCR test. This paper presents the research performed on a group of 150 cases of patients affected by neurological disorders and that were subsequently confirmed to be infected with SARS-CoV-2, which was carried out over a period of 10 months within the Neurology Department and Laboratory of Magnetic Resonance Imaging of "Sf. Andrei" Emergency Hospital in Constanta. The collected data are observational and provide perspectives on the neurological pathology associated with the SARS-CoV-2 virus, and on the frequently associated risk factors, associated comorbidities, and the ages of patients who were affected by the virus, as well as the clinical and paraclinical manifestations of the patients admitted to the hospital's neurology department.

Keywords: COVID-19; SARS-CoV-2 infection; neurological pathology; imaging investigations

Citation: Axelerad, A.D.; Muja, L.F.; Stroe, A.Z.; Zlotea, L.A.; Sirbu, C.A.; Docu Axelerad, S.; Jianu, D.C.; Frecus, C.E.; Mihai, C.M. Impact of SARS-CoV-2 Infection in Patients with Neurological Pathology. *Diagnostics* **2022**, *12*, 473. https://doi.org/10.3390/diagnostics12020473

Academic Editor: Anna Baraniak

Received: 13 January 2022
Accepted: 10 February 2022
Published: 12 February 2022

1. Introduction

The source of COVID-19, officially designated by the World Health Organization, is infection with the SARS-CoV-2 virus (severe acute respiratory syndrome coronavirus 2) [1]. Infection with the SARS-CoV-2 virus can result in a respiratory disease that mainly attacks the lungs, provoking symptoms ranging in severity from slight or moderate to

severe, including fever, headaches, fatigue, dry cough, myalgia, and diarrhea. However, recent clinical studies have indicated that this type of pathogen has a vast infectious capacity to spread into the extrapulmonary tissues, provoking multi-organ insufficiency in patients with severe impairment. The capacity of the SARS-CoV-2 infection to invade the central nervous system and the peripheral nervous system currently represents a major concern [2]. Recent studies have reported certain neurological pathologies associated with SARS-
CoV-2 virus infection. These studies have classified certain manifestations connected to the central nervous system, such as headaches, vertigo, impaired consciousness, acute cere- brovascular disease, and epilepsy, in addition to other symptoms related to the peripheral nervous system, such as hyposmia, anosmia, hypogeusia, ageusia, myalgia, and Guillain- Barre syndrome [3]. The most frequent neurological complication that has appeared in the SARS-CoV-2 infection is ischemic stroke [4].

A thoracic computed tomography (CT) scan is recommended for cases of suspected or confirmed SARS-CoV-2 infection, due to its association with major lung disease, with the aim of evaluating and following its progression. Compared to the thoracic CT-scanning, thoracic radiography (X-rays) is not as reliable, and cannot be used to identify disease symptoms in the early stages, but in moderate to advanced phases, it may indicate the evolution of acute respiratory distress. Moreover, real-time polymerase chain reaction (RT-PCR) tests are used to diagnose infection with the SARS-CoV-2 virus. Imaging investi- gations based on CT-scanning have proven useful for diagnosis, even if an initial RT-PCR test was falsely negative [5].

The study was performed within a 10-month period, between 1 October 2020, and 31 August 2021, within the Neurology Department and Laboratory of Magnetic Reso- nance Imaging of "Sf. Andrei" Emergency Hospital in Constanta. The Constanta County Emergency Clinical Hospital's Ethics Committee for Clinical Studies (registration number 34/26.11.2021) authorized this study, which followed the guidelines of the Declaration of Helsinki. Prior to enrollment, all individuals provided written informed consent.

Following subjective and objective clinical examinations, and based on paraclinical investigations, the study group consisted of 150 cases of patients admitted to the neurology department of the hospital with various neurological diseases and who were declared to be positively infected with the virus according to the presence of ribonucleic acid (RNA) SARS-CoV-2, as determined by RT-PCR test.

2. Results

The study was performed over a period of 10 months, between 1 October 2020, and 31 August 2021. The total number of cases studied was 150. In terms of the patients' sex, the number of cases was almost equal, with 76 cases of men and 74 cases of women.

2.1. Type of Neurological Disease

Table 1 shows the types and number of cases of neurological diseases presented by patients admitted to the neurology department.

2.2. Submission of Clinical and Imaging Analysis of Representative Cases of the Studied Group

2.2.1. Case 1

A 49-year-old patient presented herself to the emergency room for ascending lower- limb paresthesia and lower-limb motor deficit, with denial of any infectious episode. Upon neurological assessment at admission, the patient was conscious, cooperative, temporo- spatially oriented, presented no rolling of the neck, normal oculomotor coordination, no nystagmus, paraparesis 3–4/5, had lively osteotendinous reflexes, no bilateral strength deficit in dorsal and plantar flexion, normal deep myo-arthro-kinetic sensitivity, tactile sensitivity with T9 sensitivity level, bilateral plantar skin reflex in flexion, bilateral postural tremor in the upper limbs, and was in a state of intermittent urine retention. CT images of the brain upon admission revealed an age-appropriate appearance.

Table 1. Type of neurological disease presented by patients, number of cases, and patient age.

Type of Neurological Disease	Number of Cases	Males	Females	30–50 Years	50–70 Years	70–100 Years
Acute ischemic stroke	44	20	24	-	14	33
Subacute ischemic stroke	36	20	16	-	14	19
Acute hemorrhagic stroke	20	9	11	-	9	11
Hemorrhagic transformation after ischemic stroke	6	3	3	-	2	4
Transient ischemic attack	5	2	3	-	2	3
Vertebrobasilar syndrome	1	-	1	-	-	1
Cerebrovascular disease	6	2	4	-	3	3
Venous thrombosis	2	1	1	1	1	-
Demyelinating lesions	4	2	2	-	1	3
Sequelae lesions	3	2	1	-	-	3
Secondary determinations	5	4	1	-	2	3
Tumor formation	3	2	1	1	1	1
Myelitis	1	-	1	1	-	-
Convulsive seizures	3	2	1	-	1	2
Guillain–Barre syndrome	1	-	1	-	1	-
Paresthesia syndrome	1	-	1	1	-	-
Paraparesis	1	-	1	-	1	-
Myasthenia gravis	1	-	1	1	-	-
Multiple sclerosis	1	1	-	1	-	-
Rasmussen's encephalitis	1	1	-	1	-	-
Motor lacunar stroke	2	1	1	-	-	2
Amnestic stroke	2	1	1	1	-	1
Disc protrusion	1	1	-	-	1	-

On the first day of hospitalization, when tested for SARS-CoV-2 RNA by RT-PCR, the result was positive. On the same day of hospitalization, the patient underwent a lumbar puncture (element count 155/mm^3, albumin 310 mg/L, chlorine 128 mmol/L, and glucose 70 mg/dL) and a thoracolumbar spine MRI, which revealed thoracic intramedullary lesions suggestive of an inflammatory-infectious substrate (Figure 1).

The patient was admitted for 1 day to the neurology ward. Being a patient with confirmed SARS-CoV-2 infection, she would ordinarily be transferred to a COVID-19- supported hospital, but she refused, and contrary to medical advice, was discharged on request. During hospitalization, the patient received hydroelectrolytic rebalancing treatment, vitamin therapy, cortico-therapy, and gastric protection.

The neurological assessment at discharge found the patient to be conscious and coop- erative, with no neck roll, no nystagmus, paraparesis 3/5, live osteotendinous reflexes, to have anesthesia with T9 sensitivity level, a vibratory disorder in the lower limbs, abolished sphincteric content, and afebrile SaO_2= 94% in atmospheric air. The patient was discharged with a permeable urinary catheter and home treatment with cortico-therapy and vitamin B1 complex in combination with vitamin B6 and B12.

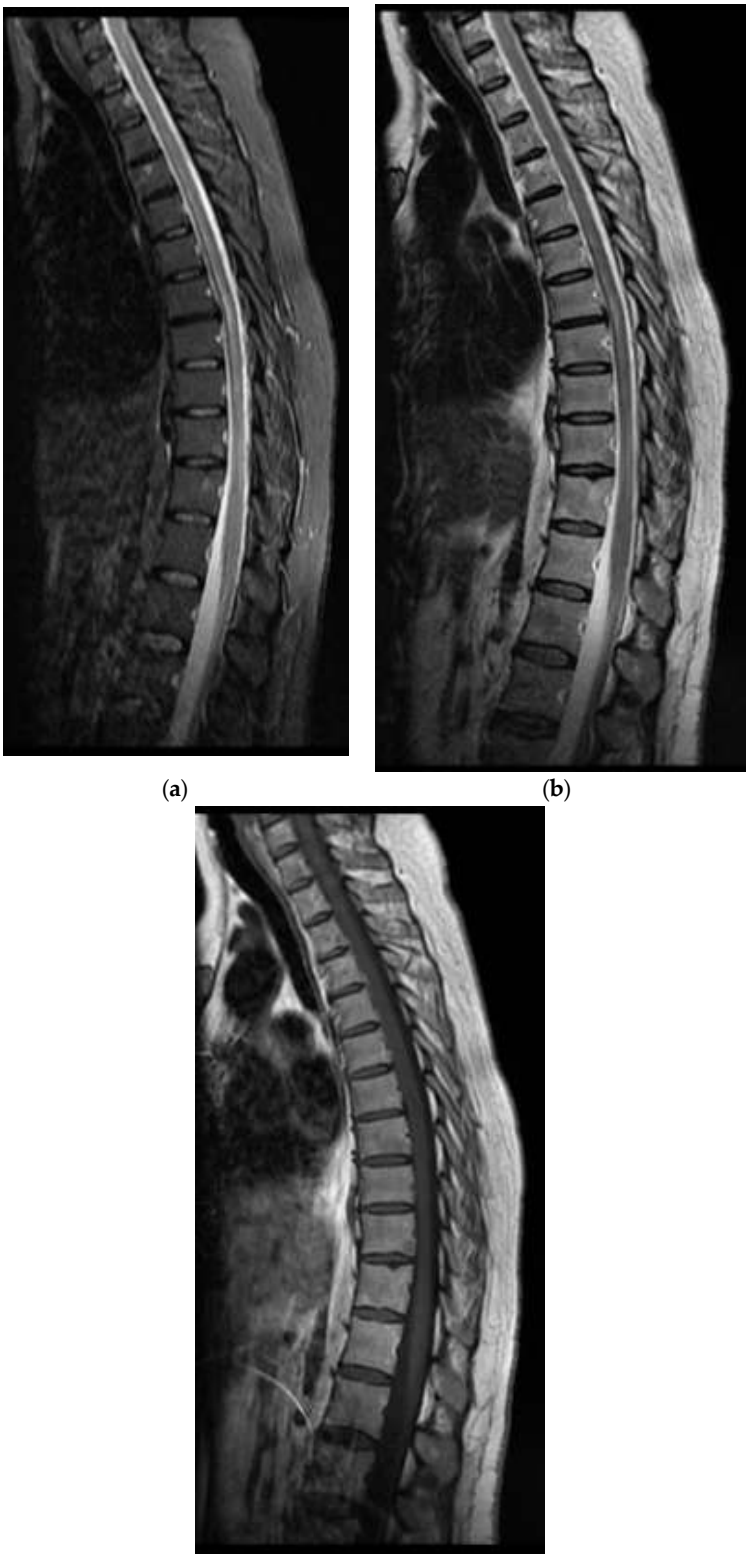
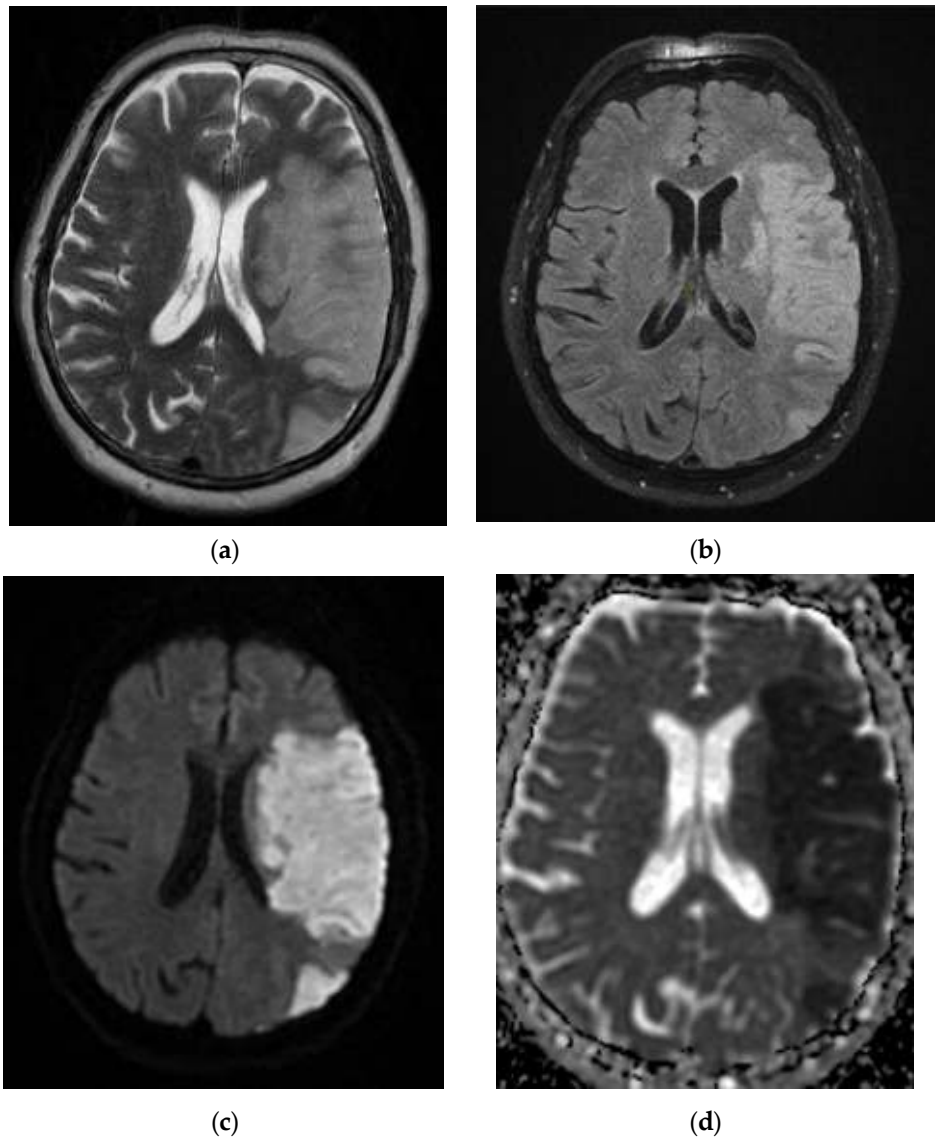


Figure 1. MRI images of the Case 1 patient show inaccurate delimited discrete hyperintense fore- shores T2-STIR (**a**,**b**), hypointense T1 (**c**), disposed at the level of lateral coordinates of the marrow corresponding to T6–T10 myeloma; thoracic intramedullary lesions with suggestive characters for an inflammatory-infectious sublayer.

2.2.2. Case 2

A 78-year-old patient with known paroxysmal atrial fibrillation in treatment, hy- pertension, and gout was admitted to the neurology department for a crisis of loss of consciousness at home, a language disorder, and a right-limb motor deficit with an un- specified onset. Upon objective neurological examination on admission, the patient was conscious, cooperative, with head and eyeballs deviated to the left, global aphasia, right hemiplegia, and right Babinski.

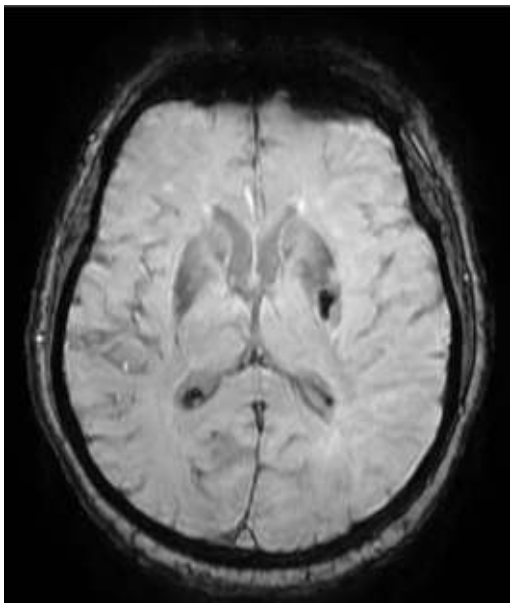
On admission, the patient had a brain CT scan, which showed acute ischemic stroke in the left MCA with an ASPECTS score of 9 points. On day 2 of hospitalization, the patient underwent a brain MRI, which showed acute ischemic stroke in the superficial and deep left Sylvian territories, in the superficial border territories of the middle cerebral artery/posterior cerebral artery, and in the middle cerebral artery/left anterior cerebral artery, with a small area of hemorrhagic transformation in the lenticular nucleus (Figure 2). On day 7 of hospitalization, the patient developed a cough with mucopurulent sputum, became trachea–bronchial loaded, and was tested by RT-PCR for SARS-CoV-2, with the result being positive. The patient was transferred to a hospital for supporting COVID-19.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. *Cont.*



(**e**)

Figure 2. MRI images of the Case 2 patient shows extended foreshores, irregularly delimited, T2- FLAIR hyperintense (**a**,**b**), with restriction of marked diffusion (**c**,**d**), confirmed by a hypointense signal on the map of apparent diffusion (**d**), situated in the cortical–subcortical frontal–parietal– occipital and left insular part, with capsular and lenticular extension. Centimetric intralesional focal signal point SWAN sequence was associated in the left lenticular nucleus (**e**). These characteristics are suggestive of acute ischemic stroke on the left superficial and profound Sylvian territory, and on the superficial territories of the border between the median cerebral artery/posterior cerebral artery and medium cerebral artery/left anterior cerebral artery (**a**–**d**), with a small area of hemorrhagic transformation in the lenticular nucleus (**e**).

2.2.3. Case 3

A 57-year-old patient with a known operated colon neoplasm, essential hypertension, and congestive heart failure was admitted for confusion syndrome and involuntary right- upper-limb movements. Objective neurological examination showed the patient to be conscious, less cooperative, and temporospatially disoriented, with apparently normal oculomotor coordination and no motor deficits; no issues were revealed with coordination and sensitivity tests.

At the time of admission to the emergency care unit, he underwent a native brain CT scan, which showed calcified atheromatous plaques located at the level of the intra- and supracavernous segments of both internal carotid arteries as well as at the level of the intracranial segment of the left vertebral artery and the right maxillary mucoceles, and a deviation of the nasal speculum with an "S" appearance, and leukoaraiosis.

On day 6, the patient underwent a native brain MRI showing multiple infra- and juxta-centimetric lesions in hyper seminal T2/FLAIR, without diffusion restriction, bilaterally arranged in the hemispheric white matter subcortical frontal–temporal–parietal, as well as in the right cerebellar hemisphere and supratentorial demyelinating lesions, most likely with ischemic vascular substrate and linear and curvilinear tracts in a SWAN (susceptibility- weighted angiography)-like manner, and left-frontal and left-parietal cortical hemosiderosis (Figure 3).

On day 10 of hospitalization, the patient underwent EEG (no epileptiform graph elements during recording; involuntary movement in the right-upper limb during inves- tigation without electrical correspondent) and brain MRI angiography with venous time determined to be within the normal limits.

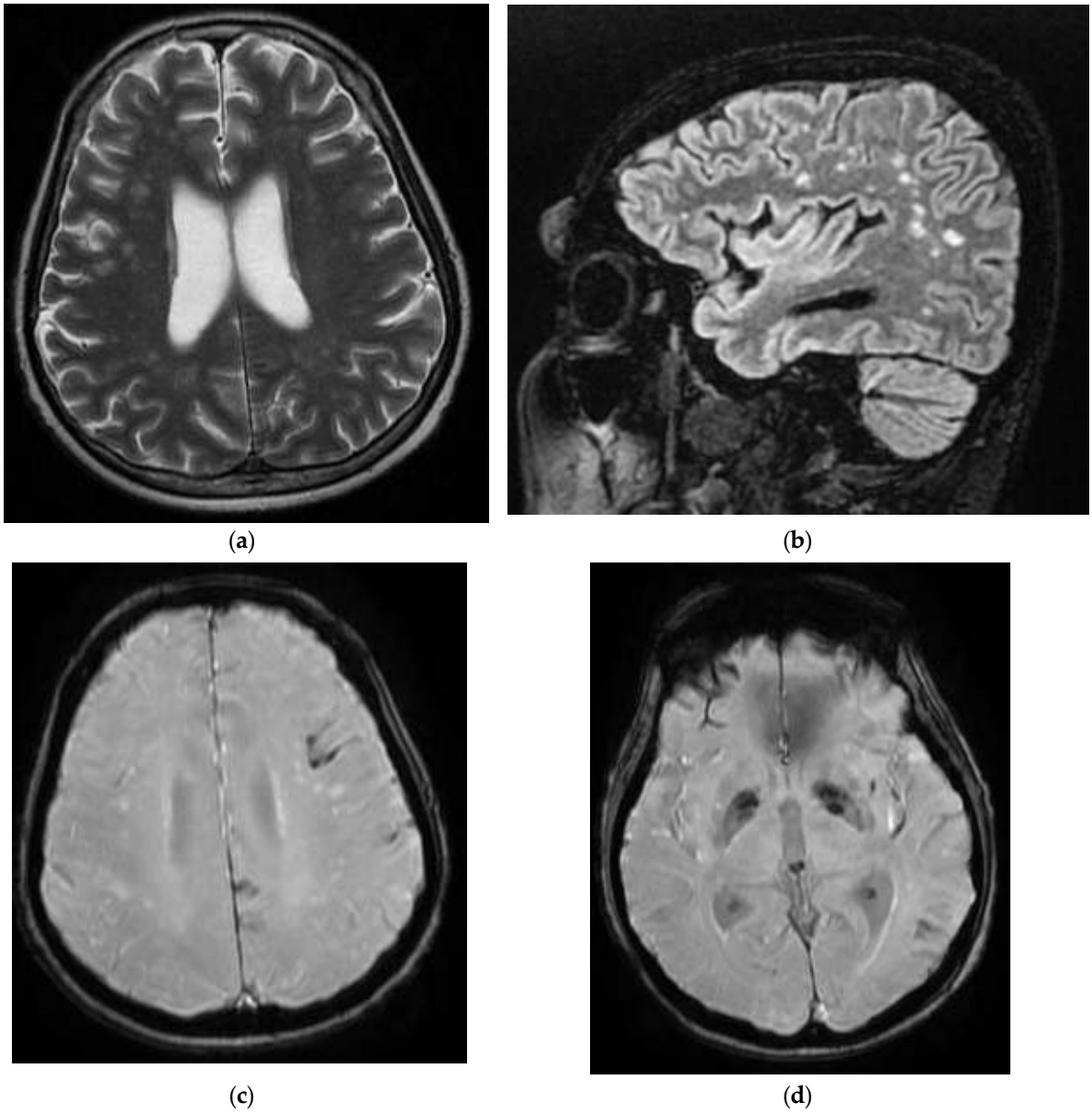


Figure 3. MRI images of the Case 3 patient reveal multiple centimetric lesions in T2/FLAIR hyper- signal (**a**,**b**), with no diffusion restriction, disposed in a white hemispheric substance in the bilateral subcortical frontal–temporal–parietal area, as well as in the right cerebellar hemisphere, and supraten- torial demyelinating lesions most probably with an ischemic vascular sublayer. Additionally, linear and curvilinear traces in SWAN signal (**c**,**d**), disposed in the left cortical parietal, frontal area–frontal cortical and left parietal hemosiderosis.

On day 11, he was tested by RT-PCR, having been in contact with a positive COVID-19 case 7 days prior and testing positive himself. During hospitalization, he received hydro- electrolytic rebalancing, and hypotensive, antiepileptic, antibiotic, analgesic, and antiemetic treatment. The patient was transferred to a COVID-19 support hospital after testing positive for SARS-CoV-2 infection.

2.2.4. Case 4

A 69-year-old patient with known type II diabetes, chronic kidney disease grade 3B, hypertension, diabetic nephropathy, and a left-thigh amputation, presented to the emer- gency care unit for right-limb motor deficit and speech impairment with onset occurring during the previous day.

Neurological objective assessment on admission showed the patient to be conscious, the head and eyeballs to be deviated to the left, with mixed aphasia, right hemiplegia, and predominantly brachial SM = 1/5 and IM = 4/5. Brain CT at the time of presentation showed supratentorial sequelae. On day 6 of hospitalization, the patient underwent a brain CT which showed an unchanged CT appearance compared to the previous examination. On day 8, he had an EEG, which showed a left frontal–temporal–occipital injury with frontal–temporal–left temporal flattening and left temporo–occipital theta waves (Figure 4).

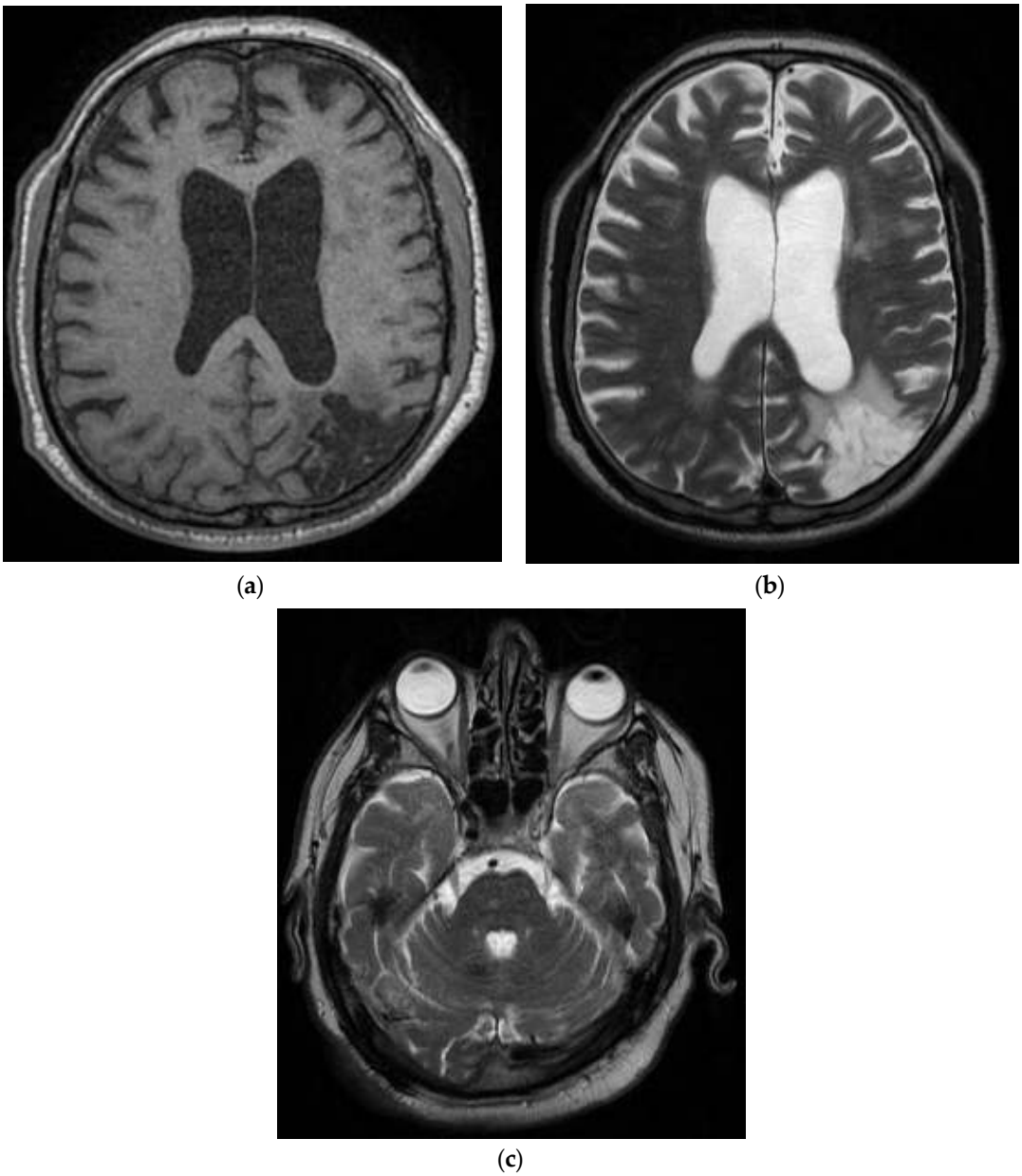


Figure 4. T1 and T2 MRI sequences in the Case 4 patient reveal left porencephalic–gliotic cortical– subcortical parietal–occipital, with a minor retractile effect on the left lateral ventricle; ischemic chronic softening on the left Sylvian territory and on the superficial territory at the border of the median cerebral artery–left posterior cerebral artery (**a**,**b**). The T2 MRI sequence shows the left internal carotid artery without a signal of circulatory flow in the intracranial segments (**c**); the rest of the big arteries located at the bases of the brain with no modifications of intraluminal signal that can be observed on the parenchymal sequences; occlusion of the left internal carotid artery (**c**).

On day 12 of hospitalization, he underwent brain MRI scanning, which revealed left frontal–parietal and left parieto–occipital cortico–subcortical porencephalic–gliotic lesions with dilatation of the adjacent interdigital spaces and a slight retractile effect on the left LV and chronic ischemic ramolitic lesions in the left superficial Sylvian territory and in the superficial border territory of the left middle cerebral artery–left posterior cerebral artery, in the left internal carotid artery with absent circulating flow signal in intracranial segments (the remaining large basal brain arteries were without detectable intralumenal signal changes on parenchymal sequences), and left internal carotid artery occlusion. On day 17 of admission, the patient was tested by RT-PCR, which showed a positive result, resulting in the patient being transferred to a COVID-19 support hospital.

2.2.5. Case 5

A 68-year-old female patient, known to have stroke sequelae and Alzheimer's disease in the dementia stage, was brought in for subtended tonic–clonic seizures. Neurological assessment on admission showed the patient to be conscious, uncooperative, to have reactive intermediate pupils, present bilateral corneal reflex, left-limb spasticity, apparent upper and lower limbs falling equally to the bed plane, showing right hemicorporeal motor Jacksonisms during consultation, and possessing sacral region decubitus scars. At the time of presentation in the emergency department, the patient presented a positive result for SARS-CoV-2 RNA as tested by RT-PCR.

Chest CT angiography and PET were carried out for the pulmonary arteries and the native brain CT showed cerebellar abiotrophy. On day 5 of hospitalization, the brain CT was repeated, which showed an unchanged appearance from the previous CT examination (cerebellar abiotrophy; in observation, corpus callosum dysgenesis). On day 10 of hospi- talization, a brain MRI was performed, which showed an imprecisely delimited area of intense T2/FLAIR signal, slightly restrictive in diffusion, located cortico-subcortically at the level of the left tonsil, late subacute infarction in the territory of the anterior choroidal artery (Figure 5).

Neurological examination at discharge showed the patient to be cachectic, conscious, cooperative, to sporadically execute simple orders and only partially respond to simple questions, with the right hemiparesis 3/5 equally distributed, left hemiparesis spastic sequelae; swallowing was possible, and sacral scars were present. Laboratory tests changed during hospitalization, showing increased D-dimers, thrombocytopenia, increased CK-MB, increased alkaline reserve, increased urea, and hypokalemia. The patient was discharged with home treatment.

2.2.6. Case 6

This patient, aged 66 years, presented to the emergency department for language disorders with fluctuating evolution. Neurological objective examination on admission showed the patient to be conscious, cooperative, with no motor deficits, lower limb ataxia, and bilateral plantar–indifferent cutaneous reflex. During hospitalization, he underwent a diabetes consultation, after which insulin therapy was initiated, a carotid Doppler echo, showing bilateral carotid atheromatosis, an EMG, which showed predominantly sensory axonal polyneuropathy, a cervical–thoracic spine MRI which showed T7–T9–T10 intraspon- gious herniation and a C5–C6 disc overhang; there was also a C6–C7 protrusion with left C7 radicular conflict, and a brain MRI evidencing T1–T2 nonhomogeneous hyperintense material partially occupying the transverse sinus and left–sided sigmoid sinus, with ex- tension to the jugular bulb–left transverse–sigmoid–jugular venous subacute thrombosis (Figure 6).

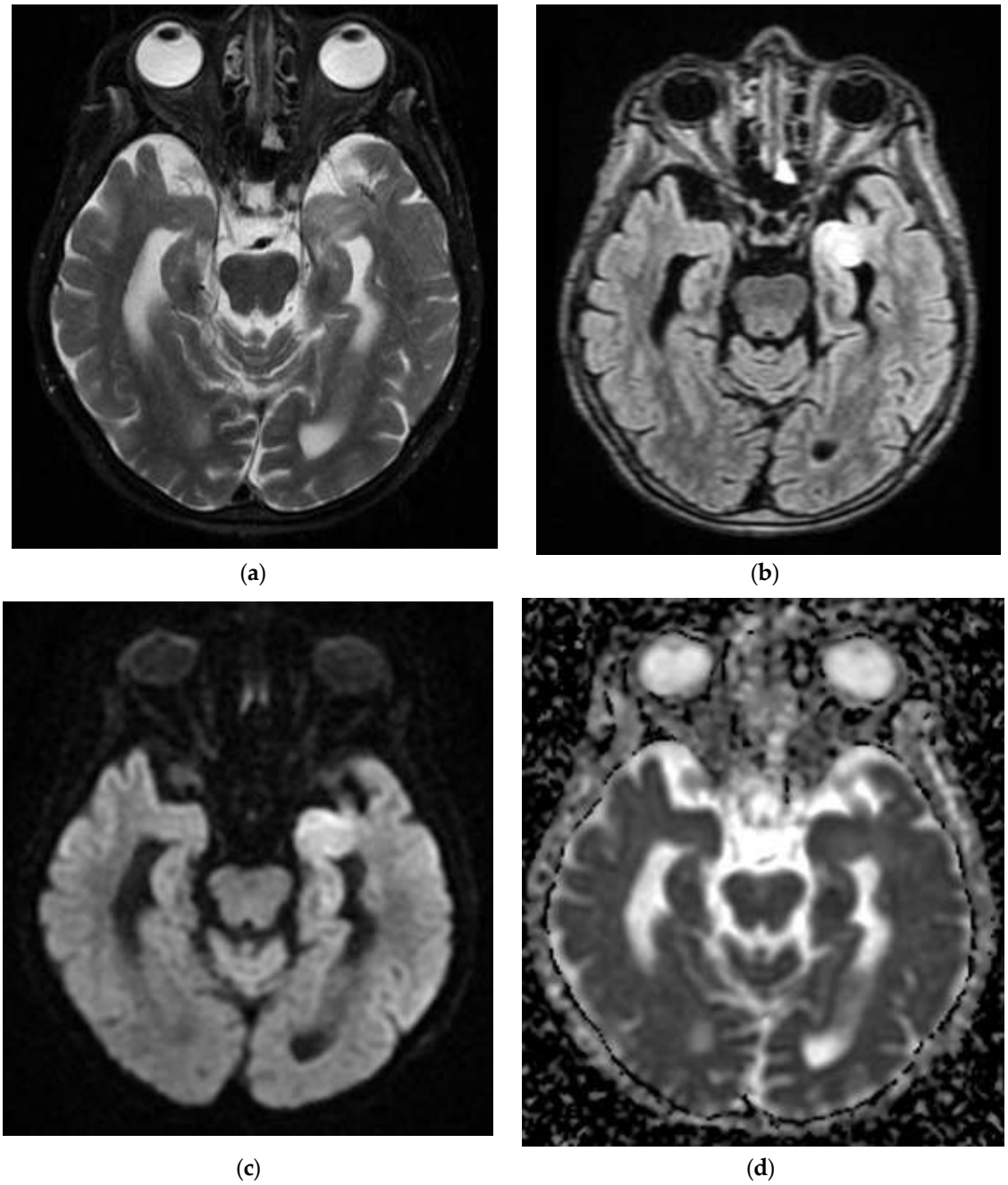
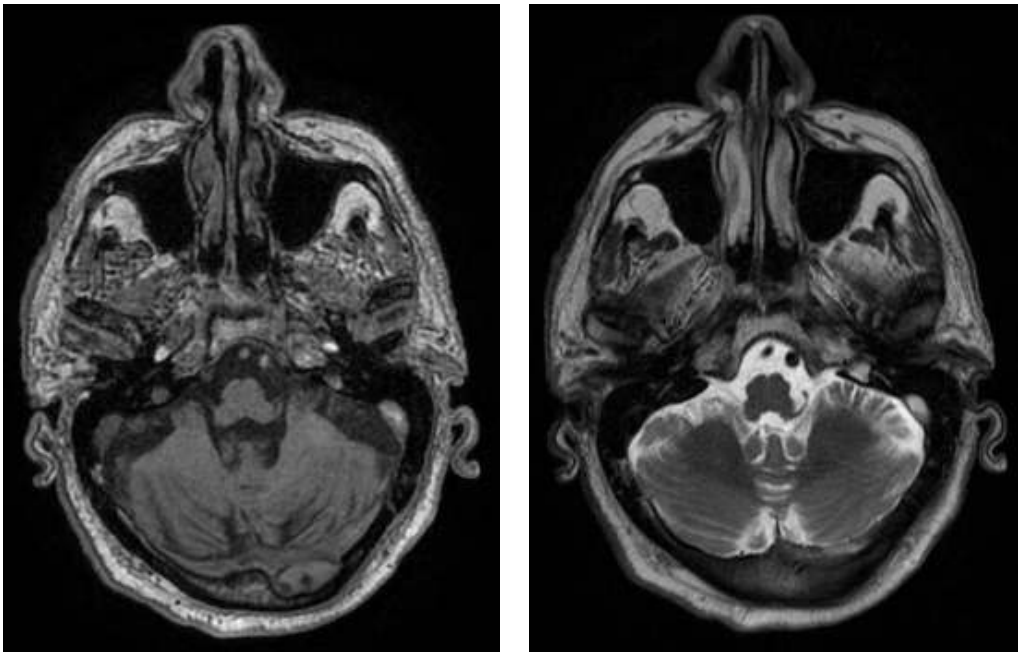
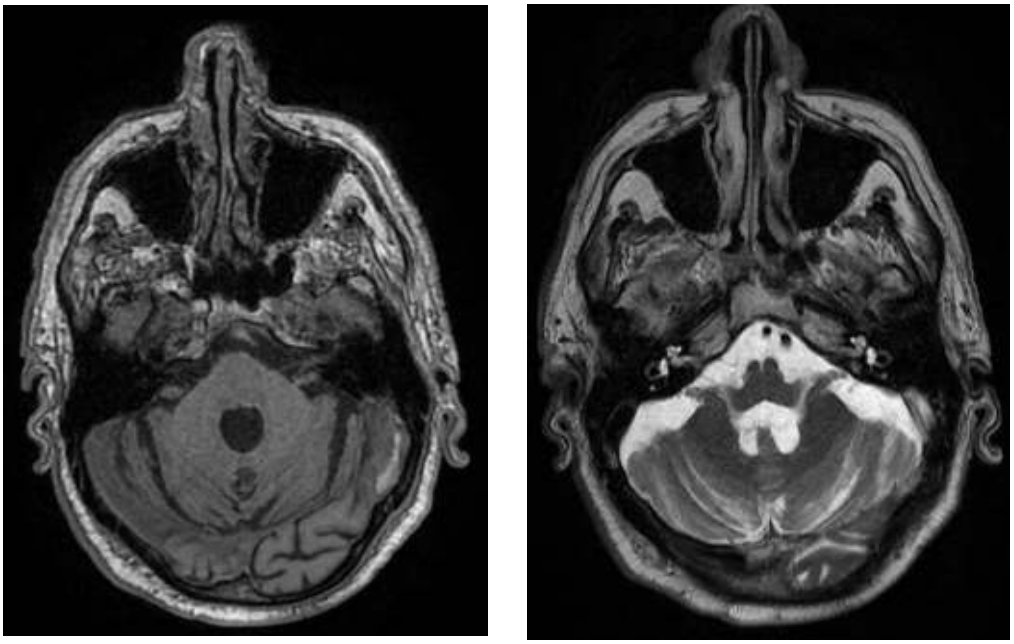


Figure 5. Cerebral MRI examination of the Case 5 patient highlights inaccurately delimited area of intense T2 and FLAIR signal (**a**,**b**), slightly restrictive in diffusion (**c**,**d**), situated in the cortical and subcortical area of the left tonsil. These images are conclusive for subacute tardive infarction on the territory of the anterior choroidal artery.

On admission, the patient had a negative RT-PCR test, but on day 16 of admission, the patient tested positive for SARS-CoV-2 RNA by RT-PCR, so the patient was transferred to the hospital providing COVID-19 support.

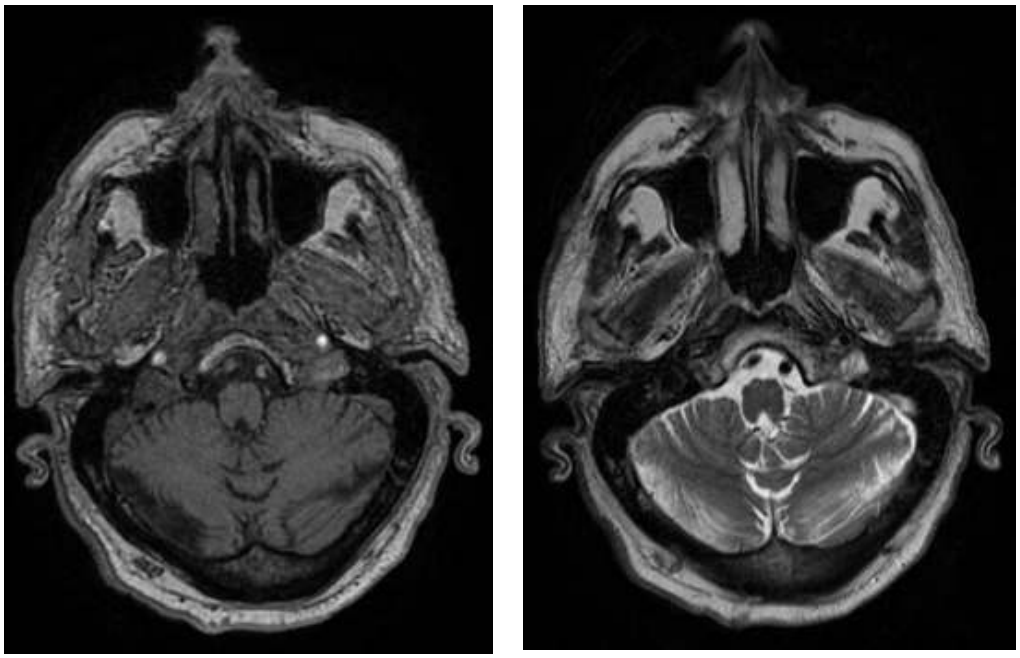


(**a**) (**b**)



(**c**) (**d**)

Figure 6. *Cont.*



(**e**) (**f**)

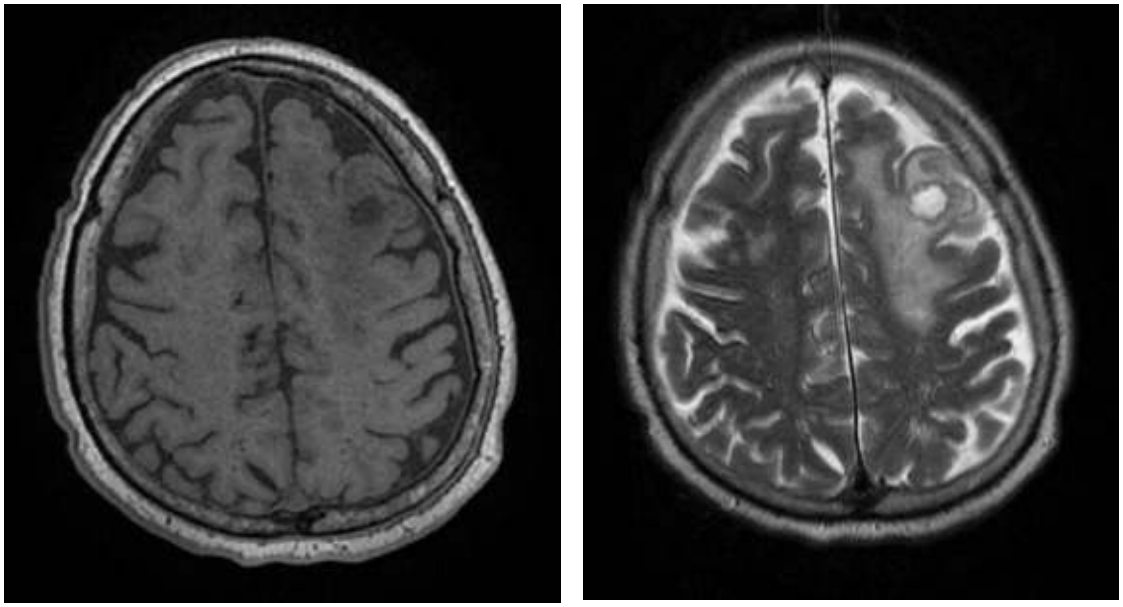
Figure 6. Hyperintense and nonhomogeneous T1 and T2 material, in the Case 6 patient, which partially occupies the transverse sinus (**a**,**b**) and left sigmoid sinus (**c**,**d**) towards the jugular bulb (**e**,**f**)–left venous transverse–sigmoid–jugular subacute thrombosis.

2.2.7. Case 7

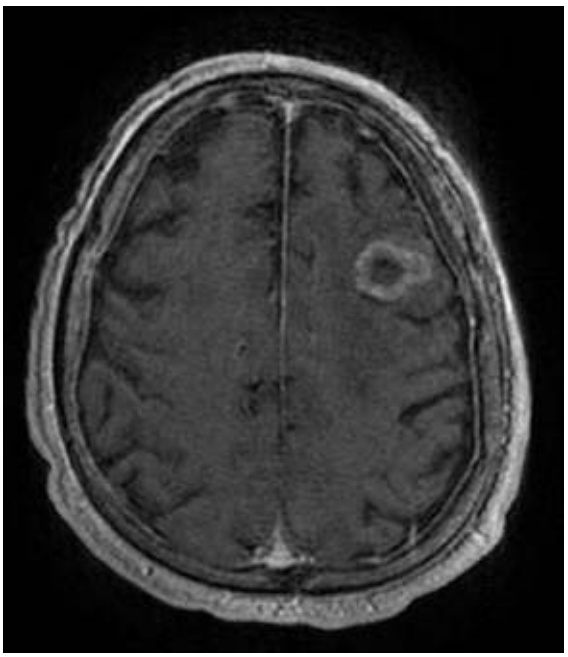
A 71-year-old patient, known to have arterial hypertension grade III basal cell epithe- lioma, presented with two generalized tonic–clonic seizures, without sphincter relaxation, without biting the tongue, for which he was admitted for further investigations and special- ist diagnosis. Neurological objective examination on admission showed the patient to be conscious, postcritical, noncooperative, with no neck roll, normal oculomotricity, preserved reflexes, equal intermediate pupils reactive to light stimuli, and no apparent motor deficits. At the time of presentation, an RT-PCR test for SARS-CoV-2 was positive, a cord– pulmonary X-ray showed bilateral apical sequelae, bilateral interstitial fibrotic changes, normal sized cord and calcifications in the aortic button; a brain CT showed a left-frontal space-replacement formation, and a secondary lesion was observed.

During hospitalization, the patient underwent native and contrast-enhanced abdomi- nal MRI, which revealed a pancreatic cephalic expansive–infiltrative formation with gastro- duodenal infiltration and locoregional adenopathy and a simple LDH cyst, and a contrast brain MRI showed a cystic solid mass with peripheral annular gadophilia, axial dimensions of 23/20 mm, extensive perilesional oedema, and a left midfrontal subcortical–left-frontal expansive lesion, most likely of secondary determination significance (Figure 7).

Biological results revealed altered tumor markers (CA 19-9 = 78.9, CEA = 9.9, NSE = 40.1), corrected hypokalemia, corrected hyponatremia, altered urinalysis, elevated cardiac en- zymes, biological inflammatory syndrome, and elevated blood glucose. During hospitaliza- tion, he received treatment with cerebral antiedematous, corticosteroid, antiplatelet, statin, gastric protector, analgesic, antiemetic, antiepileptic, myorelaxant, hypotensive, diuretic, beta blocker, and antiviral. Neurological objective assessment at discharge showed the patient to be conscious, partially temporospatially oriented, and with no motor deficit. He was discharged with antiplatelet, statin, and antiepileptic medications to be taken at home.



(**a**) (**b**)



(**c**)

Figure 7. MRI T1 (**a**), T2 (**b**), and T1 sequence with contrast injection in the Case 7 patient (**c**) show solid-cyst mass (**a**,**b**) with ring-shaped peripheral gadophilia (**c**) and extended perilesional edema (**b**), disposed in the middle-left subcortical area. These pathological images are suggestive of a left-frontal expansive lesion, most likely meaning a secondary determination.

2.2.8. Case 8

A 79-year-old female patient, known to have hypertension and insulin-dependent type II diabetes mellitus, presented to the emergency care unit initially with hypoglycemia (blood glucose = 50 mg/dL) and subsequently presented with a right-limb motor deficit, which was predominantly brachial. Neurological objective examination on admission showed the patient to be conscious, cooperative, partially temporospatially oriented, with symmetrical facies, and predominant brachial right hemiparesis. At the time of presentation, she had a brain CT scan showing a CT appearance within age limits, a cord–pulmonary X-ray showing no evolving pleuropulmonary lesions, and a negative RT-PCR test.

During hospitalization, she underwent a carotid Doppler echo, which evidenced bilateral carotid atheromatosis, and a brain MRI, which evidenced an intraventricular FLAIR intense signal beach, moderately restrictive in diffusion, located in the cortical– subcortical left superior occipital subacute infarction (Figure 8). Chest CT was performed to monitor the lung lesions and specific inflammatory samples were collected on the recommendation of the infection specialist, with whom repeated consultations were carried out to establish therapeutic management.

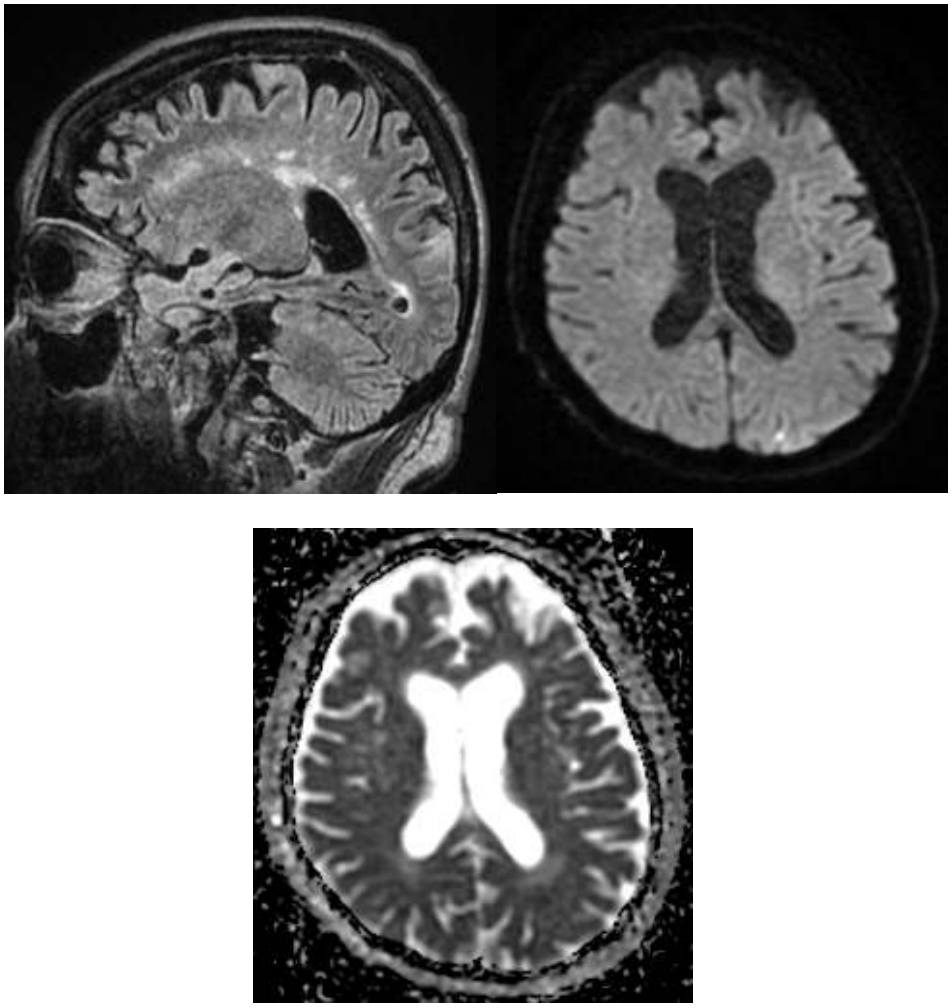


Figure 8. MRI sequences for the Case 8 patient indicate infra-centimetric foreshore of intense FLAIR signal (**a**), moderately restrictive in diffusion (**b**,**c**), situated on the left superior occipital lobe revealing subacute left superior occipital infarction.

A chest CT showed alveolo-interstitial infiltrates with a tendency to confluence in bilateral lungs, compatible with COVID-19 pneumonia image-wise, CO-RADS 6-score severity (4/25p), slight pulmonary micronodules with sequelae appearance, and vesicular lithiasis. Seven days later, the repetition of chest CT showed pleurisy in the small right pleura, bilateral dorso-basal pulmonary condensation processes compatible with atelectatic processes, alveolo-interstitial infiltrates with a tendency to consolidation compatible with COVID-19 pneumonia, CO-RADS 6 image-frameable dimensional progression from a previous CT scan, a moderate severity score (11/25p), bilateral pulmonary micronoduleswith sequelae, and gallbladder lithiasis. An RT-PCR test was conducted, and the result was positive. A biological assessment revealed mild corrected hypokalemia, UTI with treatedproteus mirabilis, hyperglycemia, and cardiac enzymes slightly altered.

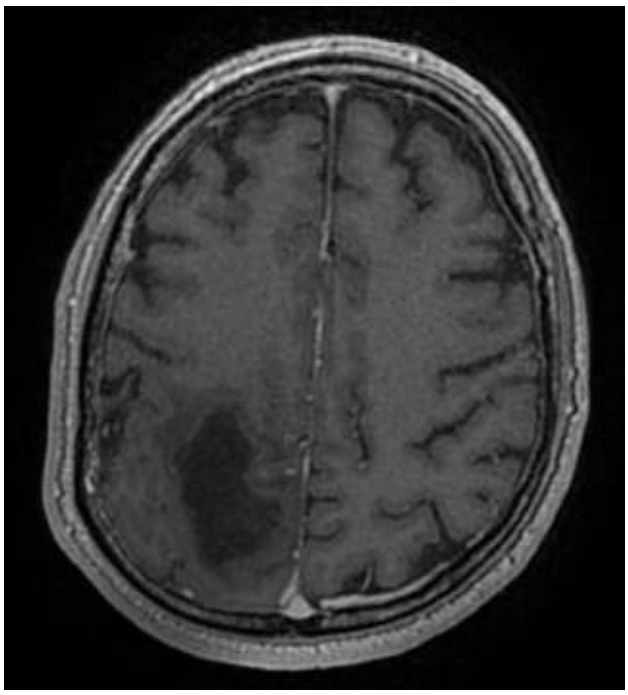
During hospitalization, the patient received treatment with cerebral depletive, antiplatelet, then
double antiplatelet, statin, hypotensive, diuretic, beta-blocker, antibiotic therapy, EU biotic, insulin, antialgesic, gastric protector, hydration solutions, antiviral treatment, corticotherapy, anticoagulant, initially HGMM, then NOAC, under which the evolution of the patient was favorable. Neurological objective examination at extenuation showed the patient to be conscious, cooperative, partially temporo-spatially oriented, with an MMSE test score = 20 points, no neck rolling, normal oculomotricity, no motor deficits,no coordination or sensitivity disorders, afebrile, no cough, and no dyspnea.

2.2.9. Case 9

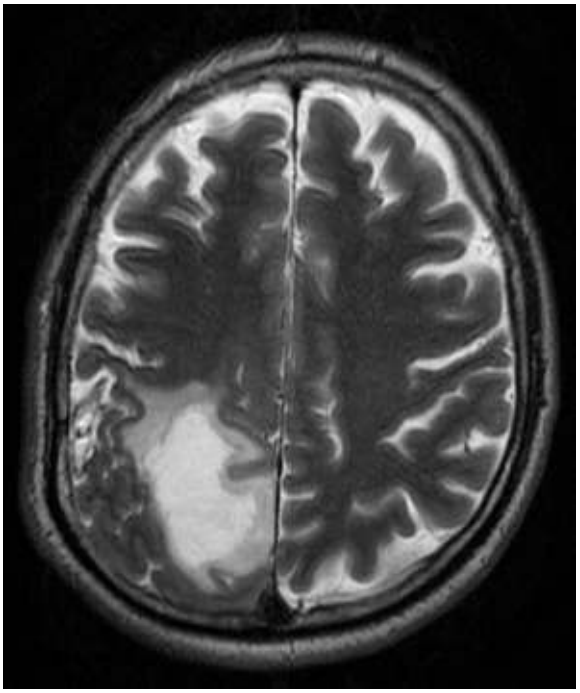
A 41-year-old patient, who was a smoker and recently discharged from a medical clinic with a diagnosis of unknown substance intoxication and metabolic acidosis, presented tothe emergency care unit due to seizures. The patient also presented himself 3 days priorwith headaches, whereby he experienced a seizure during his admission.

Neurological objective examination on admission showed the patient to be conscious, cooperative, with no neck rolling, biting tongue, nystagmus was exhaustible on right lateral gaze, normal oculomotor coordination, left hemiparetic whip 4/5, osteotendinous reflexes were present, symmetrical, ataxia on left heel–knee test. During hospitalization, native and contrast-enhanced brain CTs were performed, showing right area, parietal encephalo- malacia area, and old consolidated bilateral parietal fractures. Brain MRI showed an oval mass with dimensions of about 45/24/46 mm, isointense fluid content with cerebrospinal fluid, normal water diffusion, and negative gadophilia, located subcortically in the right parietal; the lesion was circumscribed and crossed by several very fine vascular tracts, did not communicate with the ventricles or the subarachnoid space, associated with the perilesional plaques of the intense FLAIR signal, and exerted a slight mass effect on the right lateral ventricle in the right parietal cystic expansive process (Figure 9).

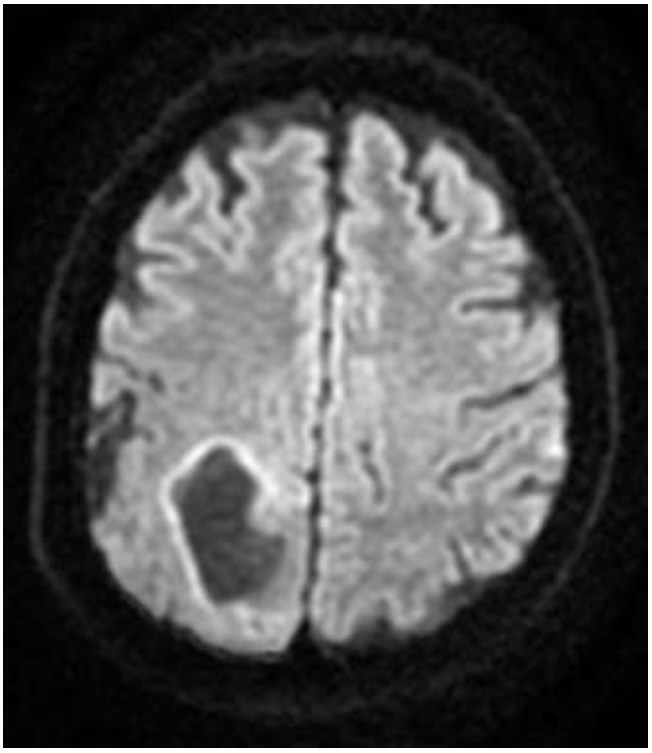
On day 10 of hospitalization, the RT-PCR test was positive. Biological changes showed discrete mixed hyperlipidemia. During hospitalization, antiepileptic and antiedema cere- bral treatment was administered. Neurological objective examination at discharge showed the patient to be conscious, cooperative, and temporospatially oriented, with no neck rolling, normal oculomotor function, no motor deficit, and no sensitivity or coordinationdisorders, and walking without support was possible.



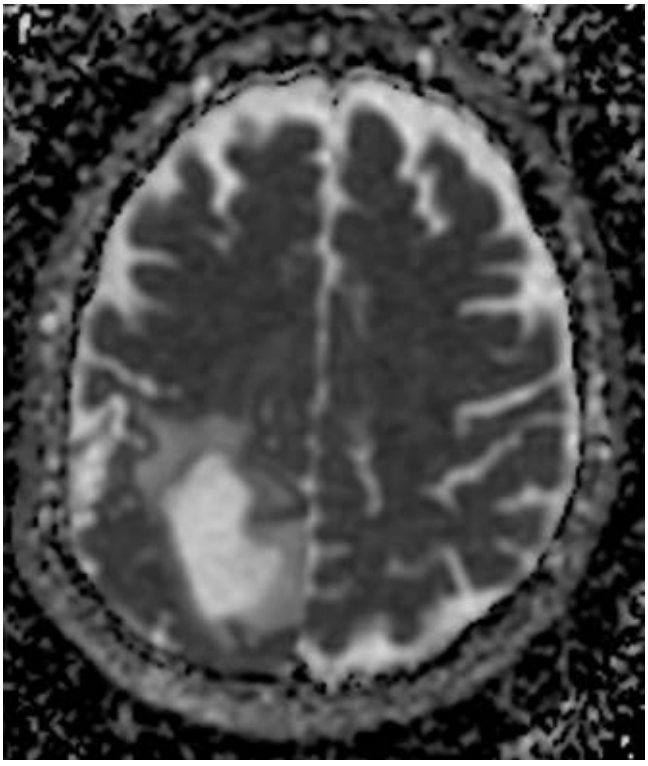
(**a**)



(**b**)

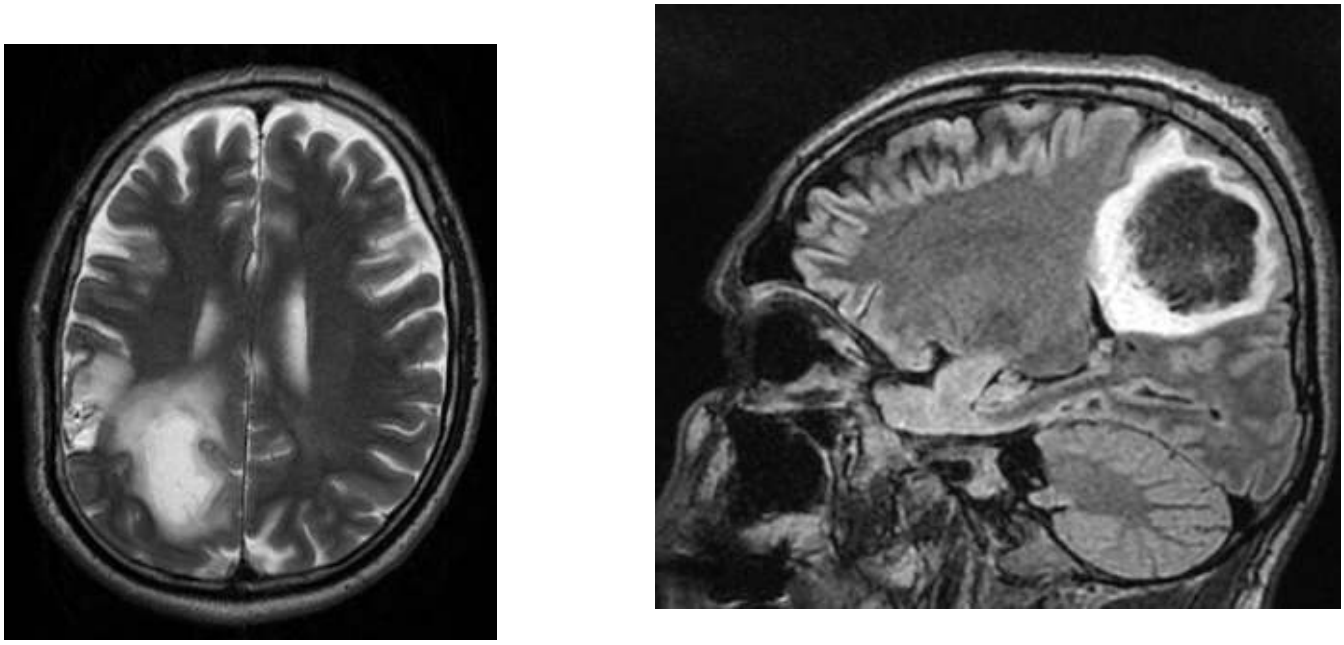


(**c**)



(**d**)

Figure 9. *Cont.*



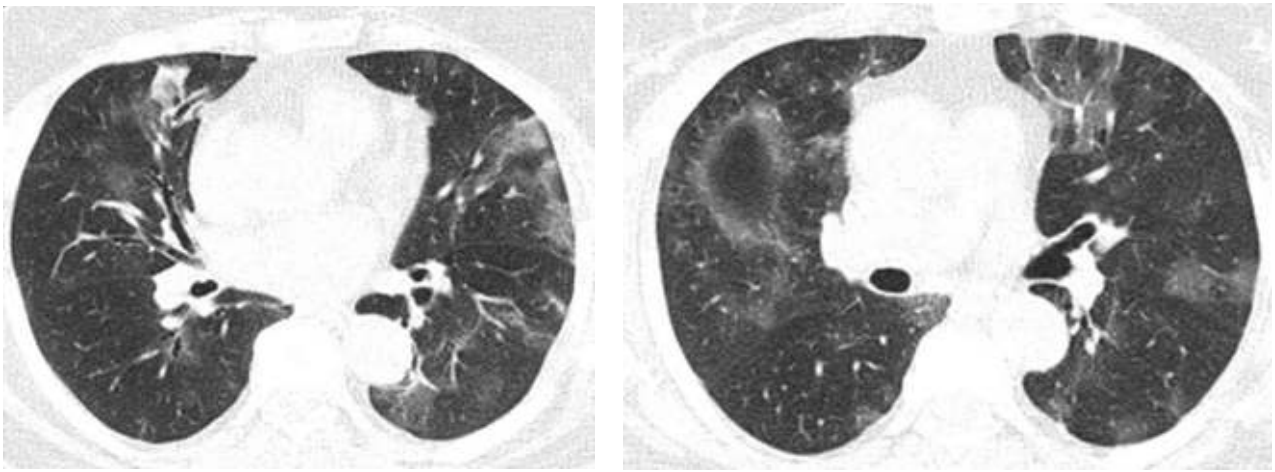
(**e**) (**f**)

Figure 9. MRI images of the Case 9 patient show oval mass with liquid content (**a**,**b**) and normal diffusion of water (**c**,**d**), located in the right parietal subcortical area; the lesion described is circum-scribed and crossed by very thin vascular lines (**e**), and does not communicate with the ventricles nor with the subarachnoid space, associates with the perilesional foreshores of the intense FLAIR signal (**f**), and has a slight mass effect on the right lateral ventricle (**e**). In conclusion, the findings revealed a right parietal cystic expansive process.

2.2.10. Case 10

A recently infected 72-year-old elderly patient with a severe form of COVID-19 was transferred from the Cardiology department with acute fibro flutter, and a recent brain CT (admission from Infectious Diseases Hospital) showed left ischemic PCA stroke appearance, with a brain MRI describing a left ischemic ACP stroke that transformed into hemorrhagic stroke, present during acute COVID-19 infection and post-hospitalization at home with bilateral lower limb plegic motor deficit.

Neurological objective assessment on admission showed the patient to be conscious, partially cooperative, and partially temporospatially oriented, with a right-upper-limb motor deficit, flaccid paraplegia, abolished osteotendinous reflexes in the lower limbs, and no tactile surface sensitivity disorders. During hospitalization, she underwent na- tive and contrast-enhanced chest CT scanning, which showed alveolo-interstitial changes suggestive of post-COVID-19 status, lumbar puncture (CSF macro- and microscopically normal appearance), and a thoracolumbar spine MRI showing a left minor split disc L5-S1 with a subligamentary nonmigrated pulposus nuclear fragment. Biological tests revealed liver cytolysis, hypokalemia, and corrected hyperglycemia. During hospitalization, she was treated with statin, antiplatelet, and hydration infusion solutions, with unfavorable evolution. The patient suffered cardio-respiratory arrest (Figures 10–14).



(a) (**b**)

Figure 10. Pulmonary CT images of the Case 10 patient show lesions with a polymorphic aspect,some under the form of inaccurately delimited areas of matt-glass clouding (**a**,**b**), and others with increased densities and associated interlobular septal thickness (**a**), randomly disposed at the level of both pulmonary fields. The tendency was to condensate some lesions from the level of the right anterior basal segment (**a**). Linear fibrosis outlines with retractile effect on the parietal pleura and on some subsegmental bronchial outlines, which could be highlighted especially at the inferior lingual level and at the level of the basal pyramid, bilaterally (**a**,**b**). In conclusion, fiber–alveolar–interstitial modifications are compatible with lesions of SARS-CoV-2 type in various phases of evolution, with a severity score of 13 (8 right lung, 5 left lung), determinable in moderate impairment.

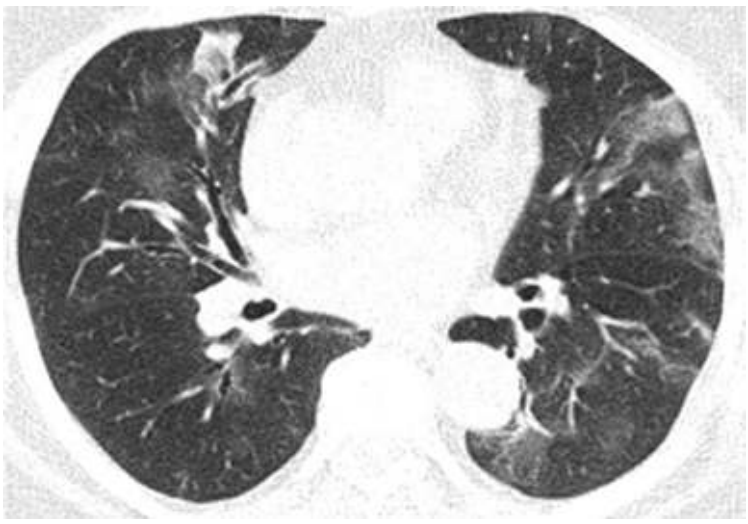


Figure 11. In evolution, CT images of the Case 10 patient show important numerical and dimensional progression of pulmonary lesions randomly distributed on more than 70% of the entire surface ofboth pulmonary fields. In conclusion, bilateral pulmonary condensations of SARS-CoV-2 type werein progress, with a severity score of 20 (13 at previous examination) and were determined to exhibit a severe degree of disorder.

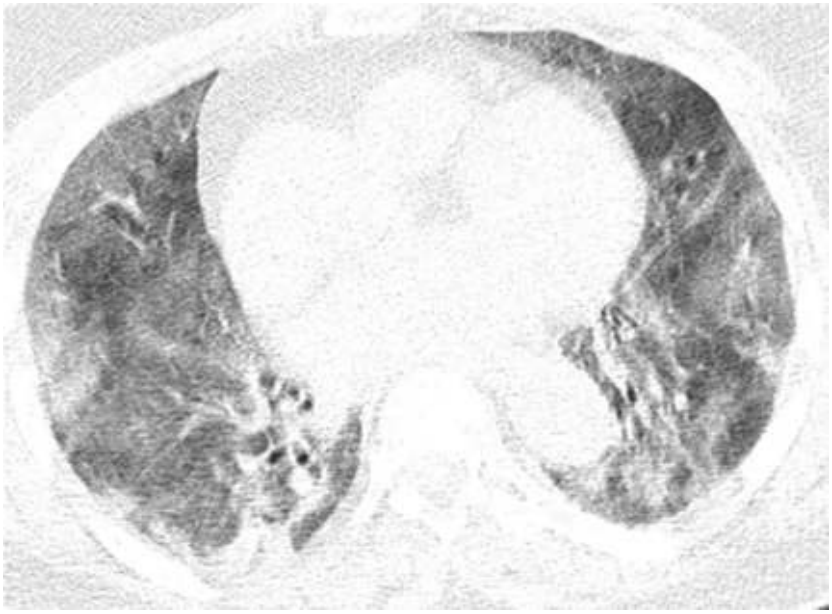


Figure 12. Four days later, pulmonary CT in the Case 10 patient showed dimensional extension of the previously described lesions, with a tendency of small-holding. In conclusion, pulmonary lesions of SARS-CoV-2 type (with the tendency of consolidation) in dimensional progression, with a severity score = 22 (20 for the previous examination), which corresponds to a severe disorder.

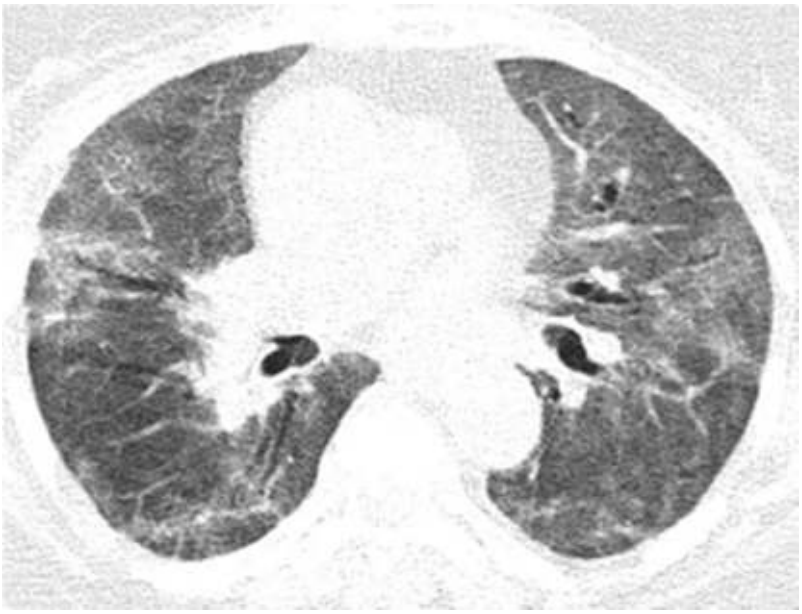


Figure 13. After 10 days, the thoraco-pulmonary CT of the Case 10 patient revealed that the previously described lesions are numerically and dimensionally stationary, at times reduced in intensity. In conclusion, pulmonary lesions of SARS-CoV-2 type in discrete remission, and were severely impaired.

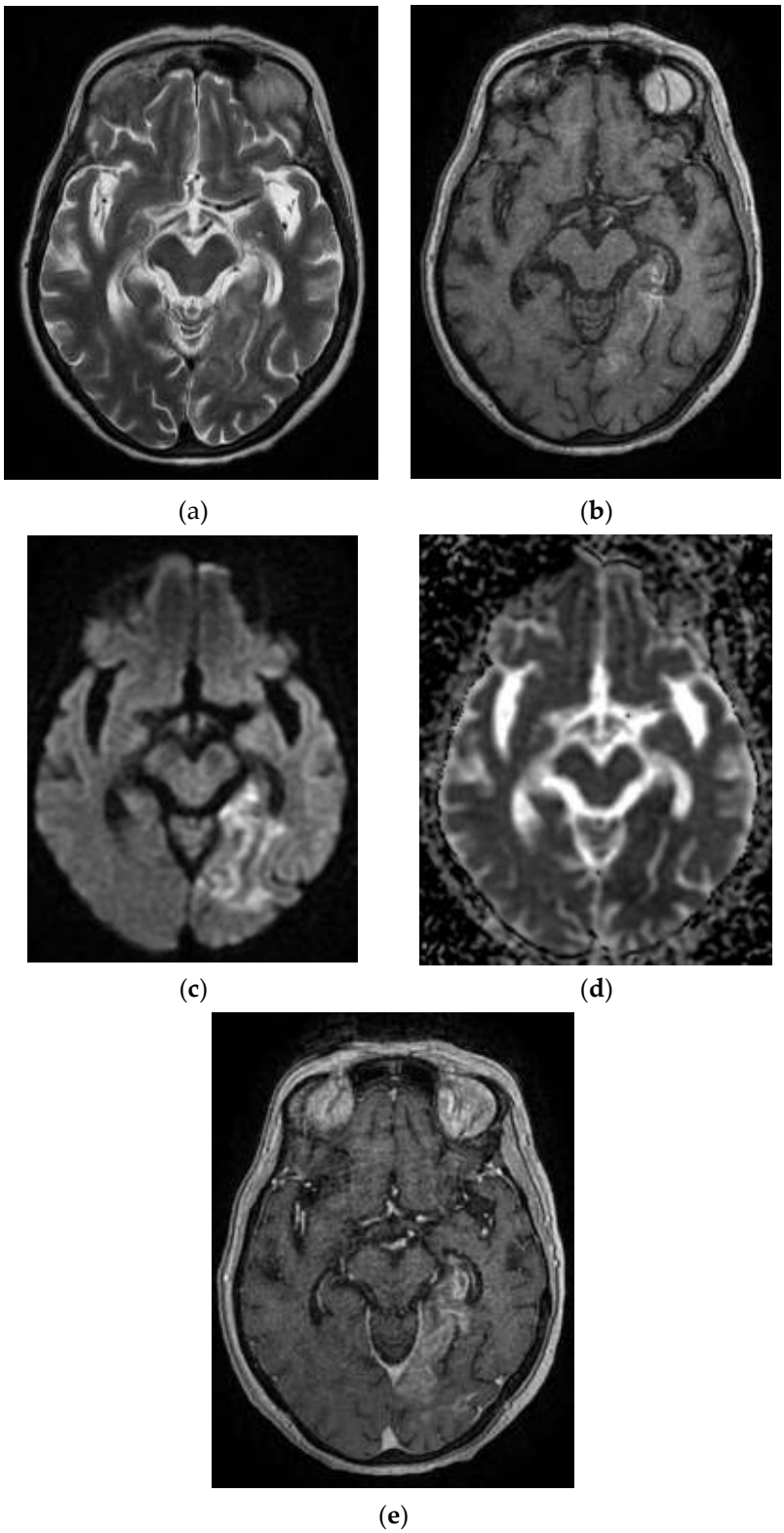


Figure 14. After 11 days from the first pulmonary CT, the cranio-cerebral MRI in the Case 10 patient showed foreshadowing of intense T2 signal (**a**), with deposits of predominantly peripheral methe- moglobin (**b**), deficient restriction of diffusion (**c**,**d**) and gyriform gadolinium I (**e**), developed in the cortical–subcortical–occipital left median side with extension in the thalamic nucleus on the same part. In conclusion, infarction hemorrhagically transformed into superficial and profound territories of the left posterior cerebral artery.

2.3. Neurological Features of Patients

Table 2 describes the baseline and clinical characteristics of COVID-19 patients with neurological features.

Table 2. Baseline and clinical characteristics of COVID-19 patients with neurological features.

Case ID	Age	Neurological Features			Other Diagnostics
		Neurological-COVID-19-related diagnostic	Other neurological diagnoses	Neurological symptoms	
1	49	Myelitis; paraparesis		Ascending lower limb paresthesia and lower limb motor deficit	Biological inflammatory syndrome; acute urinary retention; confirmed infection with SARS-CoV-2.
2	78	Acute ischemic stroke in the superficial and deep-left Sylvian territory and in the superficial border territories, middle cerebral artery/posterior cerebral artery, and middle cerebral artery/left anterior cerebral artery by cardioembolic mechanism; right hemiplegia		A crisis of loss of consciousness, language disorder, and right limb motor deficit	Permanent atrial fibrillation; hypertension; gout; biological inflammatory syndrome; interstitial pneumonia; confirmed infection with SARS-CoV-2.
3	57	Cerebrovascular disease; involuntary right upper limb movements	Cerebral atherosclerosis	Confusion syndrome and involuntary right upper limb movements	Dilated cardiomyopathy with left ventricular dysfunction; congestive heart failure class III (New York Heart Association (NYHA)); mild mitral regurgitation; acute chronickidney disease; upper rectalneoplasm operated and radio chemically treated; hypertension; confirmed infection with SARS-CoV-2.
4	69	Cerebrovascular disease	Sequelae of stroke; right sequelae hemiparesis	Right limb motor deficit and speech impairment with onset	Left internal carotid artery occlusion; hypertension; type II diabetes mellitus with diabetic nephropathy. Chronic kidney disease grade 3B; left-thigh amputation; confirmed infection with SARS-CoV-2.
5	68	Subacute ischemic stroke in the territory of the anterior choroidal artery by the most likely atherothrombotic mechanism; sub intending tonic–clonic seizures	Sequelae of stroke; left hemiparesis spastic sequelae; Alzheimer's disease; Alzheimer's dementia; cerebrovascular disease; agenesis of thecorpus callosum; cerebellar abiotrophy	Subtended tonic–clonic seizures	Confirmed infection with SARS-CoV-2.

Table 2. *Cont.*

Case ID	Age	Neurological Features			Other Diagnostics
6	66	Left transverse–sigmoid–jugular venous subacute thrombosis	Cerebrovascular disease; predominantly sensory axonal polyneuropathy; newly discovered type II diabetes; C6-C7 protrusion with left C7 radicular conflict; neurocognitive disorder	Language disorders with fluctuating evolution	Confirmed infection with SARS-CoV-2
7		Secondary epilepsy-generalized seizures	Secondary cerebral determinations	Two generalized tonic–clonic seizures, without sphincter relaxation, withoutbiting tongue	Pulmonary secondary determinations; pancreatic head tumor; sequelae of ischemic stroke left posterior cerebral artery; essential hypertension; temporospatial disorientation; confirmed infection with SARS-CoV-2
8	79	Left occipital subacute stroke by atherothrombotic mechanism	Sequelae of stroke; cerebral atheromatosis; mixed dementia	Right limb motor deficit, predominantly brachial	Bilateral carotid atheromatosis; insulin-dependent type II diabetes mellitus with poor control; diabetic polyneuropathy; confirmed infection with SARS-CoV-2
9	41	Morphic seizure.	Right parietal cystic expansive process; left parietal hemangioma; chronic left parietal microhemorrhages	Headaches and seizure during his admission	Mixed dyslipidemia; confirmed infection with SARS-CoV-2
10	72	Left ischemic posterior cerebral artery stroke transformed hemorrhagic; flaccid paraplegia; exitus by cardiorespiratory arrest	Sequelae of stroke	Bilateral lower limb plegic motor deficit	Hypertension; atrial flutter; recent post-COVID-19 condition

3. Discussion

The COVID-19 patients in our 10 case reports presented with a complex panel of neurological diagnostics, including myelitis with paraparesis, acute ischemic stroke in various territories with hemiplegia, cerebrovascular disease with involuntary movements, seizures, and left transverse–sigmoid–jugular venous subacute thrombosis.

Cerebrovascular disease, in addition to certain other neurological features, has often been associated with acute SARS-CoV-2 infection. Numerous pathophysiological mecha- nisms have been postulated to explain the SARS-CoV-2-related prothrombotic condition, as both direct and indirect consequences of the viral infection. Aside from hypercoagulable characteristics, it is hypothesized that SARS-CoV-2-related endothelitis and microangiopa- thy lead to hemorrhagic stroke. Consequently, intracranial hemorrhage in COVID-19 patients could be the result of hemorrhagic transformation of ischemic stroke, original hemorrhagic stroke, or traumatic intracranial hemorrhage.

The processes underlying the apparition of these neurological symptoms remain unknown. Numerous ideas have been advanced since SARS-CoV-2 was first detected, such as that the neuroinvasion of the virus comes from its ability to enter via the olfactory

groove or directly into the nervous system via circulation [6,7]. However, these results might be the result of secondary immunological processes and a severe inflammatory state induced by infection, or of significant hypoxia caused by critical illness and concomitantdisorders [6,7].

We have identified various neurological pathologies which can be correlated to thepositive diagnosis of the RNA SARS-CoV-2 testing by RT-PCR tests or which represent only incidental findings following a clinical or paraclinical examination by magnetic resonance imaging.

The types of disorders encountered during the study are acute stroke, subacute stroke, hemorrhagic stroke, ischemic stroke that then becomes hemorrhagic, carotid transitory ischemic stroke, vertebrobasilar insufficiency, cerebrovascular disease, venous thrombo- sis, demyelinating lesions, sequela lesions, secondary determinations, tumoral formation, myelitis, seizures, Guillain–Barre syndrome, paraesthetica syndrome, paraparesis, myasthe- nia gravis, multiple sclerosis, Rasmussen's encephalitis, movement lacunar stroke, amnestic syndrome, and disk protrusion.

The most frequent pathology was ischemia, which is strongly connected to a diagnosis of COVID-19 and is represented by cerebral strokes with various ages and types of evolu-tion, with a total number of 85 cases of cerebral strokes, 44 cases of acute cerebral strokes,36 cases of subacute cerebral strokes, and 5 cases of carotid transitory ischemic stroke. This classification might also include four cases of ischemic ictus, two cases of movement lacunar stroke, two cases of amnestic syndrome, three cases of stroke sequela, and two cases of sequela lesions. Among the patients who had been diagnosed only with the cerebrovascular disorder, we found six cases and four cases of demyelinating lesions, which might have had a vascular-ischemic sublayer. We also encountered a case of vertebrobasilar syndrome.The hemorrhagic stroke associated with the SARS-CoV-2 virus occupied a significant place in a group of 26 cases, of which 20 patients presented with a hemorrhagic acute stroke and another 6 patients presented with a hemorrhagic stroke transformed from an ischemic stroke.

Moreover, the neoplasia pathology associated with COVID-19 had substantial rep-resentation, as we found three cases with tumoral formations and five cases with sec- ondary determinations. We found two cases of venous cerebral thrombosis associated with SARS-CoV-2 infection and three cases of convulsive crisis.

The range of neurological pathologies associated with the infection of SARS-CoV-2 is vast, and we might include other pathologies here, such as myelitis, Guillain–Barre syndrome, paresthesia, paraparesis, myasthenia gravis, multiple sclerosis, Rasmussen's encephalitis, and disk protrusion.

Ghannam et al. indicated that infection with the SARS-CoV-2 virus results not only in respiratory disease, and that neurological complications are frequently experienced. Associated neurological pathologies are often encountered, including ischemic and hem- orrhagic strokes, Guillain–Barre syndrome, encephalitis, and convulsions. In a studio located in Wuhan, China, out of 214 cases with SARS-CoV-2 infection, 78 had neurological complications. Patients with severe SARS-CoV-2 infection have a higher risk of developing neurological complications. The manifestations point to the central nervous system and peripheral nervous system, and symptoms such as headaches, vertigo, muscular weakness, sensory alteration, and impaired consciousness may also appear [8].

As part of the classification according to age groups, it was noticed that the greatestnumber of cases were of patients aged 70–80 years, representing 58 out of the 150 cases thatwere evaluated.

This age group was followed by 60–70-year-olds, with 40 cases, followed by the 80–90-year-old age group, in which we found 26 cases. In these first three groups, is- chemic vascular pathology was most frequently presented. Next, we found the group of 50–60-year-olds, with 17 cases, where inflammatory pathologies and venous thrombosis were the most frequently presented.

For the other age intervals, the number of cases decreased dramatically, with one case for the group of 30–40 years, seven cases for 40–50 years, and one case for 90–100 years.

Sullivan et al. evaluated neurological pathologies according to patient age, individual age, or the age interval of the group, with the aim of classifying the frequency of neuro- logical complications associated with SARS-CoV-2 and that affect children (under the age of 19 years), young adults (between 19 and 50 years), and adults (over the age of 50). The patients with an average age between 60 and 69 years presented the greatest number of associated neurological disorders, and the patients aged equal to or under 9 years presented the lowest association. Patients over the age of 50 years presented the greatest number of cerebrovascular disorders. The most frequent associated neurological pathology was observed in over half of the patients in this group [9].

Patients with COVID-19 displayed a predominance of respiratory and nervous system symptoms. Among several patients of the latter, there were complaints of loss of smell and taste, ataxia, and impairment to peripheral nervous system, which might reflect the SARS-CoV-2 virus presenting with neurotoxicity.

Six key clock genes, including *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1,* and *CRY2*, control circadian rhythm. Clock genes are involved in the regulation of metabolic and immunolog- ical responses, including the release of pro-inflammatory interleukins. As a result, lifestyle modifications, including adjustments in the light regime, a reduction in the amplitude of room temperature, a change in the timing of eating, and the allocation of a food according to its calorific value throughout the day, contribute to metabolic abnormalities and the appari- tion of a low-intensity systemic inflammatory process [10]. Additionally, the circadian clock governs fundamental bodily processes, including lung capacity and sleeping as well as activities in the neural tissue that contribute to neurological and psychiatric illnesses, such as auto-aggression and neuropathic pain. Additionally, several studies have discovered a link between the development of certain symptoms and particular circadian chronotypes that might aid in the creation of chronotherapy and enhance therapy by administering medication in line with the patient's circadian rhythm [11,12].

Sleep phase abnormalities were the most prevalent circadian rhythm abnormalities. Boiko et al. [11] discovered that COVID-19 infection in antecedents increased patients' susceptibility to developing circadian rhythm abnormalities, including delayed sleep phase disorder. Additionally, it was shown that individuals with COVID-19 exhibit elevated levels of trait and state anxiety. The high incidence of delayed sleep phase disorder might be a result of both the neurotropic properties of SARS-CoV-2 and isolation linked with the COVID-19 outbreak. Therefore, the impact of a modification in light exposure on everyday activities was detected under lockdown settings, meaning a change of the rhythm by three or more hours. This has resulted in decreased exposure to natural light, which has a detrimental effect on the main circadian oscillator, as well as physical and nutritional activities. Amongst circadian rhythm abnormalities, delayed sleep phase disturbance is most prevalent in the post-COVID-19 era and is related to an increased degree of anxiety in these individuals.

Global Impact of the COVID-19 Pandemic on Different Areas in Low- and Middle-Income Countries

Formerly, it was thought that the SARS-CoV-2 virus was limited to the respiratory system, mostly impacting the lungs; nevertheless, new investigations have demonstrated the virus's multisystem impact, most notably impacting brain tissue. COVID-19 illness may indeed be associated with a variety of mental disorders, including post-traumatic stress disorder, obsessive–compulsive disorder, anxiety, delirium, and depression, either directly or indirectly. It has the potential to exacerbate pre-existing mental health problems or to precipitate the genesis of new psychiatric diseases [13]. Increasing COVID-19 instances, elevated illness burden, and a lack of social support may all contribute to short-term mental health concerns, while economic losses also present because mandated lockdowns could have a long-term effect on people's mental health [14].

Due to a global scarcity of frontline staff dedicated to containing the epidemic, several governments have reassigned psychiatrists to critical care settings to handle COVID-19 patients. As a result, mental hospital outpatient departments were closed, resulting in a massive increase in mental health difficulties in some countries. In these cases, imposed lockdowns and restrictive restrictions regarding physical isolation have robbed many patients of access to essential mental health treatment [15].

The COVID-19 pandemic has highlighted underdeveloped health systems in low- and middle-income countries (LMICs) and significant treatment disparities, at least in the area of mental health. As a result of these issues, individuals with severe mental illness die earlier, have more physical ailments, and receive less medical treatment than the general population [16].

Additionally, it is vital to educate the public in low- and middle-income countries on the acknowledgment of mental health problems as diseases, the importance of social and familial assistance, and the importance of avoiding social stigma of those who suffer from mental health disorders.

Most neurological symptoms have been shown to develop early throughout the course of the disease (in some studies, the median time to hospital admission was 1–2 days) [15,16]. Several individuals lacking classic COVID-19 manifestations (fever, coughing, anorexia, and diarrhea) presented to the hospital with solely neurological manifestations, as in our study. As a result of the impact of SARS-CoV-2 infection on neurological diseases, we must closely monitor patients with COVID-19 for neurological symptoms, particularly those with serious infections that could have led to their mortality. Additionally, during the COVID-19 epidemic era, clinicians must include SARS-CoV-2 infection as a differential diagnosis whenever meeting patients with these neurological signs to minimize late or incorrect diagnosis and to limit the spread.

The purpose of this research was to provide a complete assessment of neurologi- cal symptoms related with SARS-CoV-2 infection and to detail the course of disease and outcomes of COVID-19 individuals who developed neurological symptoms in 10 representative patients from Constanta Clinical County Hospital, which is situated in a low–middle-income country. This work may provide critical new clinical information on COVID-19, assisting physicians in raising awareness of its association with neurological symptoms and diagnosis of COVID-19 infection in low- and middle-income countries.

It is particularly significant to note that, in our study, contrary to other studies, the patients firstly presented with neurological features, and COVID-19 infection was subse-quently discovered. Moreover, quick clinical decline or aggravation may be accompanied with a neurological event, including stroke in patients with severe COVID-19, contributing to the disease's high mortality rate. Additionally, doctors might include SARS-CoV-2 infection as a differential diagnosis when meeting patients with these neurological signs during the COVID-19 pandemic period to prevent late diagnosis or misdiagnosis as well as to limit the spread. To facilitate future clinical care, additional precise epidemiological data and further pathophysiology findings are required.

Global restrictions imposed to prevent and control the spread of a new COVID-19 wave resulted in a financial crisis in LMICs, restricting access to food and other basic requirements due to border closures. The combined consequences of poverty, climate change, and the COVID-19 epidemic have exacerbated food insecurity in some LMICs. COVID-19, thus, worsened an already-existing food crisis in these countries due to the imposition of government restrictions and lockdown measures that restricted work options and income.

Food insecurity is a major socioeconomic and public health problem in low- and middle-income nations. It is associated with adverse health effects and a reduction in self- reported health status, decreased micronutrient intake, fruit and vegetable consumption, weight gain, and birth abnormalities. As with challenges experienced by other resource- related issues (e.g., housing instability, energy uncertainty), inequality and poverty might exacerbate nutritional deficiencies, illness, and disease management. Individuals who

experience poverty have much worse health outcomes and less access to healthcare than those who do not. Poor nutrition may exacerbate pre-existing illnesses, such as inadequate glucose control in diabetic patients, final renal disease in patients with chronic kidney disease, as well as affect the treatment of other chronic diseases. COVID-19's presence in LMICs has restricted access to healthcare and impacted attempts to treat, diagnose, immunize, and monitor other infectious diseases. Food instability may further aggravate health problems and expenditures for families with children that have specific healthcare requirements, or for persons with disabilities.

A number of examples can be provided depicting the impact of the COVID-19 pan- demic on LMICs.

Afghanistan's continuous struggle has created several difficulties for the country's population. Afghanistan has seen a significant rise in food shortfalls because of its reliance on neighboring nations during the epidemic [17]. Individuals attain food security when they have continuous physical and economic access to an adequate supply of safe and nutritious food that meets their dietary demands and preferences. Food scarcity, political unrest, and the third wave of COVID-19 have made it impossible to obtain basic supplies.Consequently, folks are forced to contend with the COVID-19 pandemic amid economic collapse and despair. At this crucial point, worldwide efforts are essential to ameliorate food security.

The growth in the number of instances of the illness has the potential to overwhelm the health system, as does noncompliance with social distance measures and the introduction of variants of concern in LMICs. This rise in the transmission curve may also create conditions favorable for the emergence of further changes in the virus' structure and DNA. As a result, genomic monitoring methods are essential to detect and describe these variants as well as to determine if the vaccines against the virus that are currently in use are efficacious.

The development of efficient and dependable infectious disease monitoring systems is critical for establishing a high-quality public healthcare system and reducing the mortality rates in low- and middle-income countries. Monitoring helps facilitate the accessibility of records and knowledge and decreases the burden and propagation of unfavorable healthcare events. This allows for a rapid response in public health, efficient implementation of methods and countermeasures, and a review of suggested treatments, the rapid detection of new illnesses, promoting health security and stability for people living in LMICs.

Healthcare workers (HCWs) have been critical in containing the pandemic and miti-gating its effects. Increased working hours and frequent exposure to critically ill patients have major consequences for the health and wellbeing of physicians, which have previously been disregarded.

Infectious disease epidemics have always posed a threat to public health, especially in Africa, where outbreaks have exploded in recent years [18]. Although several infectious diseases have emerged in Africa, such as Ebola and certain other epidemic-prone infections, insufficient focus has been placed on the development of health surveillance systems. The inadequacy of the region's healthcare monitoring techniques have only recently been identified. Africa suffers from a shifting epidemiology of disease, a deficient healthcare system, and a scarcity of resources. Only a vigilant monitoring system can ensure that the best use of its available resources are made in an effective and strategically managed manner. Measures are needed to rapidly detect potential public health threats. This could be accomplished via the use of appropriate, efficient, and lengthy surveillance methods.

Dengue fever is a serious public health concern in Africa [18] and the COVID-19 pandemic has exacerbated this concern. COVID-19 accelerates the transmission of a variety of illnesses, including Zika, yellow fever, measles, mucormycosis, Lassa fever, and HIV, as has been seen in several nations on the African continent. The limits imposed in response to the COVID-19 pandemic have resulted in the suspension of vector management initiatives that aid in the management of these diseases. To avert further public health disasters, urgent and interdisciplinary measures to dengue fever epidemic management in Africannations are necessary.

COVID-19 cases have impoverished Nigeria's healthcare system and resulted in additional neglect of persons suffering with mental illness [18]. In general, there is a demand for equitable access to healthcare resources, but there is a need for adequate attention and treatment for mental health patients, which is rising in Nigeria.

The COVID-19 epidemic has hit public health emergencies in Bangladesh, a low– middle-income nation in South Asia. The resulting surge of sickness cases may generate an overburdening of the health system. This rise in the contagion curve may also encourage further alterations in the virus' structure and DNA. It is important to find, monitor, and characterize these polymorphisms and determine the efficacy of existing vaccinations against these variants.

India's healthcare sector is suffering significant difficulties as a consequence of the lack of resources to combat the COVID-19 pandemic, with HCWs also suffering the conse-quences [19]. There is an urgent need to address these flaws in the healthcare system to provide a consistent and ongoing supply of high-quality treatments in India.

4. Conclusions

The neurological manifestations associated with SARS-CoV-2 virus infection are fre-quent, with ischemic stroke being the most common, followed by hemorrhagic stroke and neoplasia pathology. In this study, we included primary tumoral formations or sec- ondary determinations. In a small number of cases, other disorders were identified, such as myelitis, convulsive crisis, Guillain–Barre syndrome, paresthesia, paraparesis, myasthenia gravis, multiple sclerosis, and Rasmussen's encephalitis. These could be related to the inflammatory response or even the hypercoagulability caused by the virus, or simply to the virus's cytopathic effect.

Another conclusion we can draw from the study is that the most affected age group was found to be between 70 and 80 years, representing 58 of the total 150 cases studied, followed by the 60–70 age group, with a total number of 40 cases, followed by the 80–90 year-old age group, with 26 cases.

COVID-19 patients often have neurological symptoms. Throughout the duration of the COVID-19 epidemic, health professionals must consider acute respiratory syndrome SARS-CoV-2 infection as a differential diagnosis in the cases of patients with neurological symptoms to minimize postponed or incorrect diagnosis and loss of the opportunity to prevent and control additional transmission.

The study is limited and observational. More studies aimed at clarifying the neurolog- ical complications associated with the infection with the SARS-CoV-2 virus are needed to establish the physiopathological mechanisms.

Author Contributions: Conceptualization, A.D.A., L.F.M. and A.Z.S.; Data curation, A.D.A. and L.F.M.; Formal analysis, A.D.A. and L.F.M.; Funding acquisition, A.D.A. and L.F.M.; Investigation, A.D.A., L.F.M., A.Z.S. and L.A.Z.; Methodology, A.D.A. and L.F.M.; Project administration, A.D.A., L.F.M. and C.M.M.; Resources, A.D.A. and L.F.M.; Software, A.D.A., L.F.M., L.A.Z. and C.M.M.; Supervision, A.D.A. and L.F.M.; Validation, A.D.A. and L.F.M.; Visualization, A.D.A., L.F.M. and A.Z.S.; Writing—original draft, A.D.A., L.F.M., A.Z.S., C.A.S., S.D.A., D.C.J., C.E.F. and C.M.M.; Writing—review and editing, A.D.A., L.F.M., A.Z.S. and C.M.M., A.D.A. and L.F.M. have equal contributions. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Sf Apostol Andrei Clinical County Hospital of Constanta (protocol code 34/26.11.2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Third-party data restrictions apply to the availability of these data. The data were obtained from Constanta's Sf Apostol Andrei County Emergency Clinical Hospital and are available from the authors with the permission of the Institutional Ethics Committee of Clinical Studies of the Constanta's Sf. Apostol Andrei County Emergency Clinical Hospital.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization (WHO). 2019. Available online: https://www.who.int/ (accessed on 27 December 2021).
2. Guadarrama-Ortiz, P.; Choreño-Parra, J.A.; Sánchez-Martínez, C.M.; Pacheco-Sánchez, F.J.; Rodríguez-Nava, A.I.; García-Quintero, G. Neurological Aspects of SARS-CoV-2 Infection: Mechanisms and Manifestations. *Front. Neurol.* **2020**, *11*. [CrossRef]
3. Niazkar, H.R.; Zibaee, B.; Nasimi, A.; Bahri, N. The neurological manifestations of COVID-19: A review article. *Neurol. Sci.* **2020**, *41*, 1667–1671. [CrossRef]
4. Abouhashem, S.; Eldawoody, H.; Taha, M.M. Cerebral venous sinus thrombosis in patients with COVID-19 infection. *Interdiscip.Neurosurg.* **2021**, *24*, 101091. [CrossRef] [PubMed]
5. Salehi, S.; Abedi, A.; Balakrishnan, S.; Gholamrezanezhad, A. Coronavirus Disease 2019 (COVID-19): A Systematic Review of Imaging Findings in 919 Patients. *Am. J. Roentgenol.* **2020**, *215*, 87–93. [CrossRef]
6. Zubair, A.S.; McAlpine, L.S.; Gardin, T.; Farhadian, S.; Kuruvilla, D.E.; Spudich, S. Neuropathogenesis and Neurologic Manifesta- tions of the Coronaviruses in the Age of Coronavirus Disease 2019: A Review. *JAMA Neurol.* **2020**, *77*, 1018–1027. [CrossRef] [PubMed]
7. Favas, T.T.; Dev, P.; Chaurasia, R.N.; Chakravarty, K.; Mishra, R.; Joshi, D.; Mishra, V.N.; Kumar, A.; Singh, V.K.; Pandey, M.; et al. Neurological manifestations of COVID-19: A systematic review and meta-analysis of proportions. *Neurol. Sci.* **2020**, *41*, 3437–3470. [CrossRef] [PubMed]
8. Ghannam, M.; Alshaer, Q.; Al-Chalabi, M.; Zakarna, L.; Robertson, J.; Manousakis, G. Neurological involvement of coronavirus disease 2019: A systematic review. *J. Neurol.* **2020**, *267*, 3135–3153. [CrossRef] [PubMed]
9. Sullivan, B.N.; Fischer, T. Age-Associated Neurological Complications of COVID-19: A Systematic Review and Meta-Analysis. *Front. Aging Neurosci.* **2021**, *13*, 653694. [CrossRef] [PubMed]
10. Kaidashev, I. The role of the circadian molecular clock in the pathogenesis of the metabolic syndrome. *Endokrynologia* **2020**, *25*, 158–170. [CrossRef]
11. Boiko, D.I.; Zhyvotovska, L.V.; Sonnik, G.T.; Skrypni;kov, A.M. Clinical and Psychopathological Characteris-tics of the Autoagres-sive Behavior in Patients with the First Psychotic Episode with Considering Circadian Rhythms. *Wiad. Lek.* **2017**, *70*, 553–557. [PubMed]
12. Burish, M.J.; Chen, Z.; Yoo, S.-H. Emerging relevance of circadian rhythms in headaches and neuropathic pain. *Acta Physiol.* **2019**, *225*, e13161. [CrossRef] [PubMed]

13. Ojeahere, M.I.; de Filippis, R.; Ransing, R.; Karaliuniene, R.; Ullah, I.; Bytyçi, D.G.; Abbass, Z.; Kilic, O.; Nahidi, M.; Hayatudeen, N.; et al. Management of psychiatric conditions and delirium during the COVID-19 pandemic across continents: Lessons learned and recommendations. *Brain Behav. Immun. Health* **2020**, *9*, 100147. [CrossRef] [PubMed]
14. Semo, B.-W.; Frissa, S.M. The Mental Health Impact of the COVID-19 Pandemic: Implications for Sub-Saharan Africa. *Psychol. Res. Behav. Manag.* **2020**, *13*, 713–720. [CrossRef] [PubMed]
15. Adiukwu, F.; Orsolini, L.; Bytyçi, D.G.; El Hayek, S.; Gonzalez-Diaz, J.M.; Larnaout, A.; Grandinetti, P.; Nofal, M.; Pereira-Sanchez, V.; Pinto da Costa, M.; et al. COVID-19 mental health care toolkit: An international collaborative effort by Early Career Psychiatrists section. *Gen. Psychiatry* **2020**, *33*, e100270. [CrossRef] [PubMed]
16. Kola, L.; Kohrt, B.A.; Hanlon, C.; Naslund, J.A.; Sikander, S.; Balaji, M.; Benjet, C.; Cheung, E.Y.L.; Eaton, J.; Gonsalves, P.; et al. COVID-19 mental health impact and responses in low-income and middle-income countries: Reimagining global mental health. *Lancet Psychiatry* **2021**, *8*, 535–550. [CrossRef]
17. Islam, Z.; Kokash, D.M.; Babar, M.S.; Uday, U.; Hasan, M.M.; Rackimuthu, S.; Essar, M.Y.; Nemat, A. Food Security, Conflict, and COVID-19: Perspective from Afghanistan. *Am. J. Trop. Med. Hyg.* **2022**, *106*, 21–24. [CrossRef] [PubMed]
18. Aborode, A.T.; Hasan, M.M.; Jain, S.; Okereke, M.; Adedeji, O.J.; Karra-Aly, A.; Fasawe, A.S. Impact of poor disease surveillance system on COVID-19 response in africa: Time to rethink and rebuilt. *Clin. Epidemiol. Glob. Health* **2021**, *12*, 100841. [CrossRef][PubMed]
19. Ghosh, S.; Moledina, N.; Hasan, M.M.; Jain, S.; Ghosh, A. Colossal challenges to healthcare workers combating the second wave of coronavirus disease 2019 (COVID-19) in India. *Infect. Control Hosp. Epidemiol.* **2021**, *2*, 1–2. [CrossRef] [PubMed]

ealthcare

MDPI

Article

Clinical Characteristics and Laboratory Findings in Children with Multisystem Inflammatory Syndrome (MIS-C)—A Retrospective Study of a Tertiary Care Center from Constanta, Romania

Cristina Maria Mihai [1,2], Tatiana Chisnoiu [1,2], Adriana Luminita Balasa [1,2], Corina Elena Frecus [1,2],
Larisia Mihai [1,2], Alexandru Cosmin Pantazi [1,2], Alina Zorina Stuparu [3,4,*] and Any Axelerad [3,4]

[1] Department of Pediatrics, General Medicine Faculty, Ovidius University, 900470 Constanta, Romania
[2] Department of Pediatrics, County Clinical Emergency Hospital of Constanta, 900591 Constanta, Romania
[3] Department of Neurology, General Medicine Faculty, Ovidius University, 900470 Constanta, Romania
[4] Department of Neurology, County Clinical Emergency Hospital of Constanta, 900591 Constanta, Romania
* Correspondence: zorina.stroe@yahoo.com; Tel.: +40-727987950

Abstract: A new hyper-inflammatory syndrome in children was identified after SARS-CoV-2 infection as a post-infectious complication that is temporally associated with coronavirus disease (COVID-19). Fever, rash, conjunctival hyperemia, and gastrointestinal problems are all clinical manifestations of multisystem inflammatory syndrome in children. This condition, in some cases, causes multisystem involvement, affecting multiple organ systems and necessitating admission to a pediatric intensive care unit. Due to limited clinical studies, it is necessary to analyze the characteristics of the pathology in order to improve the management and long-term follow-up of high-risk patients. The objective of the study was to analyze the clinical and paraclinical characteristics of children diagnosed with MIS-C. The clinical study is a retrospective, observational, descriptive research work that includes patients diagnosed with MIS-C, temporally associated with coronavirus disease, and it contains clinical characteristics, laboratory data, and demographic information. The majority of patients had normal or slightly increased leukocyte counts, which were associated with neutrophilia, lymphocytopenia, and significantly elevated inflammatory markers, including high levels of C-reactive protein, fibrinogen, the erythrocyte sedimentation rate, serum ferritin, and IL 6 and elevated levels of the cardiac enzymes NT-proBNP and D-dimers, owing to the cardiovascular system involvement in the pro-inflammatory process. At the same time, renal system involvement led to raised creatinine and high proteinuria in association with hypoalbuminemia. This characteristic of the pro-inflammatory status as well as multisystem impairment are highly suggestive of the post-infection immunological reaction of the multisystem syndrome temporally associated with SARS-CoV-2 infection.

Keywords: COVID-19 infection; multisystem inflammatory syndrome; hyperinflammation

Citation: Mihai, C.M.; Chisnoiu, T.; Balasa, A.L.; Frecus, C.E.; Mihai, L.; Pantazi, A.C.; Stuparu, A.Z.; Axelerad, A. Clinical Characteristics and Laboratory Findings in Childrenwith Multisystem Inflammatory Syndrome (MIS-C)—A RetrospectiveStudy of a Tertiary Care Center fromConstanta, Romania. *Healthcare* **2023**, *11*, 544. https://doi.org/10.3390/healthcare11040544

Academic Editor: Tao-Hsin Tung

Received: 16 December 2022
Revised: 8 February 2023
Accepted: 9 February 2023
Published: 12 February 2023

1. Introduction

"Kawasaki-like" syndrome was the first term because it presented clinical manifesta- tions like Kawasaki disease. Since then, it has been referred to as multisystem inflammatory syndrome in children (MIS-C), or PIMS-TS (pediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 infection), which typically manifests between four and six weeks after an SARS infection in a child. These terms have all been used to describe the multisystem inflammatory syndrome in children [1,2].

Three prominent explanations exist regarding the mechanisms of MIS-C and postu- late an autoimmune reaction after infection. Individuals with MIS-C appear many weeks following

acute infection with reduced viremia, decreased immunoglobulin M (IgM) levels, and elevated IgG and IgA levels [3]. As with other viral causes of autoimmunity,it is probable that SARS-CoV-2 initiates an autoimmune reaction that results in tissue destruction [4,5]. The prolonged presence of the SARS-CoV-2 antigen, which causes an immunological response that

is not well controlled, is the subject of a second theory. Yonker et al. demonstrated that, weeks after acute infection, the SARS-CoV-2 antigen remains detectable in the digestive system of individuals who acquired MIS-C [6]. Lastly, analo- gous to patients experiencing toxic shock syndrome, a third explanation proposes that the "superantigen-like activity of the SARS-CoV-2 spike protein" induces an aberrant immunereaction with cytokine storming [4]. In terms of cardiovascular impairment, it is well established that the production of cytokines (Tumor necrosis factor-alpha, IL-1, IL-2, IL-6) is a recognized cause of left ventricular failure, impacting the extracellular components and myocardial cells' force of contraction [7].

Laboratory results indicating the hyperinflammation process response include high values of C reactive protein, fibrinogen, the erythrocyte sedimentation rate, serum ferritin, and D-dimers, which are suggestive of the pathogenesis of MIS-C [2]. In some cases, coagulation disorders were described, with elevated values of D-dimers, prolonged PT, and PTT, which determines hyperfibrinogenemia [8]. It is suggested and effective to employ first-line therapy consisting of intravenous immunoglobulins (IVIG), corticotherapy, and aspirin; nevertheless, there are certain instances that do not respond to this treatment [9].

A common onset of clinical characteristics described in MIS-C is represented by fever, which is persistent for more than three days and is associated with other manifestations such as dermatological (rash, conjunctival hyperemia) or respiratory symptoms. In some cases, patients reported having digestive symptoms such as nausea, vomiting, diarrhea, or discomfort in the stomach area [10]. They also reported having cardiac involvement such as myocardial dysfunction, coronary aneurysms, or pericarditis, in addition to having elevated levels of troponin T and N-terminal pro-brain natriuretic peptide (NT-proBNP) [10]. Symptoms related to the cardiovascular system are the ones that are seen most often in patients with MIS-C. In patients with MIS-C, cardiac biomarkers including NT-pro- BNP and troponin levels are abnormally increased, which suggests heart failure and myocardial damage.

Patients may have both acute and subacute consequences of the disease, with moder- ate to severe cardiovascular symptoms, despite the fact that it is unclear what the long-term repercussions of MIS-C will be for the cardiovascular system. New research indicates thata host immune reaction, high levels of the activation of innate immune mechanisms, a massive increase in the proinflammatory cytokine, dysregulated thromboinflammation, thrombotic angiopathy, and endothelial dysfunction could be implicated in the develop- ment of COVID-19-related myocardial damage [11].

2. Materials and Methods

The research that was conducted in the Pediatrics Tertiary Care Center of the Constanta County Emergency Hospital was a retrospective, observational, descriptive study that lasted for a period of 18 months (May 2020 to October 2021). It included 35 patientswho were diagnosed with MIS-C, following clinical characteristics, laboratory data, and demographic information. There were 13 male patients and 22 female patients in the study. The statistical study was carried out using Excel and version 26 of the SPSS IBM program (v. 2010). We used Kolmogorov–Smirnoff tests, *t*-tests, and Fisher's Exact tests in our descriptive statistics. For the purposes of the statistical analysis, the information gleaned from the data was presented in the form of absolute numbers and percentages for
categorical variables. The threshold of significance was a p value less than 0.05.

The main objective of the study was to analyze the clinical characteristics and labo- ratory findings of MIS-C, considering the diagnosis, treatment, and long-term follow-up of patients.

Based on recent worldwide studies and guidelines, we suspected MIS-C and per- formed extensive laboratory tests for differential diagnosis, such as other viral and bacterial infections. Most patients had Kawasaki-like clinical manifestations at the time of admission, with persistent fever and rash.

Laboratory data were gathered, as well as evidence of SARS-CoV-2 infection, using IgG anti-SARS-CoV-2 antibodies (method: microparticle chemiluminescence, CMIA), treatment, and evolution.

Because of the large number of patients who were admitted to the department in sucha short amount of time, it was decided to carry out the clinical monitoring of patients as well as laboratory tests in accordance with the recommendations that were already decided and the protocol that was established internally.

2.1. Consent and Ethics

In the Pediatrics Clinic of the Constanta County Emergency Hospital, the research was carried out between the months of May 2020 and October 2021. The present study was approved by the Ethics Committee of the Constanta Clinical Hospital in Romania (no.13/11 April 2022), and all of the parents or legal guardians were required to fill out a written informed permission form. The principles outlined in the Declaration of Helsinki were adhered to during the course of this research (ethical considerations).

2.2. Criteria for Inclusion and Exclusion

Participants who met the parameters of the CDC case definition for MIS-C and pre- sented with multisystemic and hyperinflammatory states were considered for inclusion in the study. These participants had signs, symptomatology, and high laboratory results. One of the requirements for participation was the demonstration of a positive reaction to the SARS-CoV-2 IgG nucleocapsid and anti-spike antibodies.

2.3. Patient Information

There were 35 patients hospitalized at the Pediatric Department, ranging in age from 9 months to 15 years. Thirteen of the patients were female, making up 37% of the total, while twenty-two of the patients were male, making up 62% of the total. On the SARS- CoV-2 rapid-antigen test, the patients all had negative results. At the time of admission, 3 patients tested positive for the infection using the RT-PCR method, whereas 32 patients had negative results from the test. All of the patients tested positive for significant levels of the SARS-CoV-2 IgG nucleocapsid and anti-spike antibodies when they were put throughserological testing.

3. Results

The onset of dermatological involvement characterized by polymorphous rash (11% females and 19% males, $p = 0.03$), conjunctival hyperemia (11% females and 19% males, $p = 0.03$), or erythematous mucosa (18% females and 26% males, $p = 0.01$) was present in 83.4% of patients ($p = 0.01$), with progressive remission within the first days of treatment. In the studied group, the days with fever ranged from 5 to 10, with a maximal tempera- ture from 38 ˚C to 40.1 ˚C. Furthermore, patients showed improvement in inflammatory markers and system involvement in the first two weeks of treatment. There were no deaths. Additionally, the creatinine and albumin levels normalized.

In order to distinguish the diagnosis between MIS-C and Kawasaki disease, we ob- served the presence of thrombocytopenia, moderate lymphocytopenia, and neutrophilia in our patients. In addition, MIS-C is characterized by increased D-dimer levels ($p = 0.01$)and high NT-proBNP values ($p = 0.02$) in the laboratory. These findings are attributable to the involvement of the heart in the condition characterized by a hyper-inflammatory immune response.

Gastrointestinal symptoms represented by nausea, vomiting, and diarrhea were asso- ciated with elevated levels of fecal calprotectin in three patients.

At the onset, it was observed that most patients had normal or slightly increased leukocyte numbers ($p = 0.02$) (Figure 1A), associated with neutrophilia ($p = 0.04$) (Figure 1B) and lymphocytopenia ($p = 0.02$) (Figure 1C), thrombocytopenia (Figure 1D) and significantly elevated inflammatory markers, with high values of C-reactive protein (CRP) ($p = 0.02$)

(Figure 1E), fibrinogen ($p = 0.03$), the erythrocyte sedimentation rate ($p = 0.03$) (Figure 2A), and serum ferritin ($p = 0.05$) (Figure 2B) and elevated values of the cardiac enzymes NT- proBNP ($p < 0.001$) and D-dimers ($p = 0.01$) due to the cardiovascular system involvement in the pro-inflammatory process. There was a significant correlation between high values of CRP and the presence of lower levels of serum albumin ($p = 0.02$), as well as higher levels of fibrinogen ($p = 0.03$), serum ferritin ($p = 0.04$), and D-dimer ($p = 0.02$).

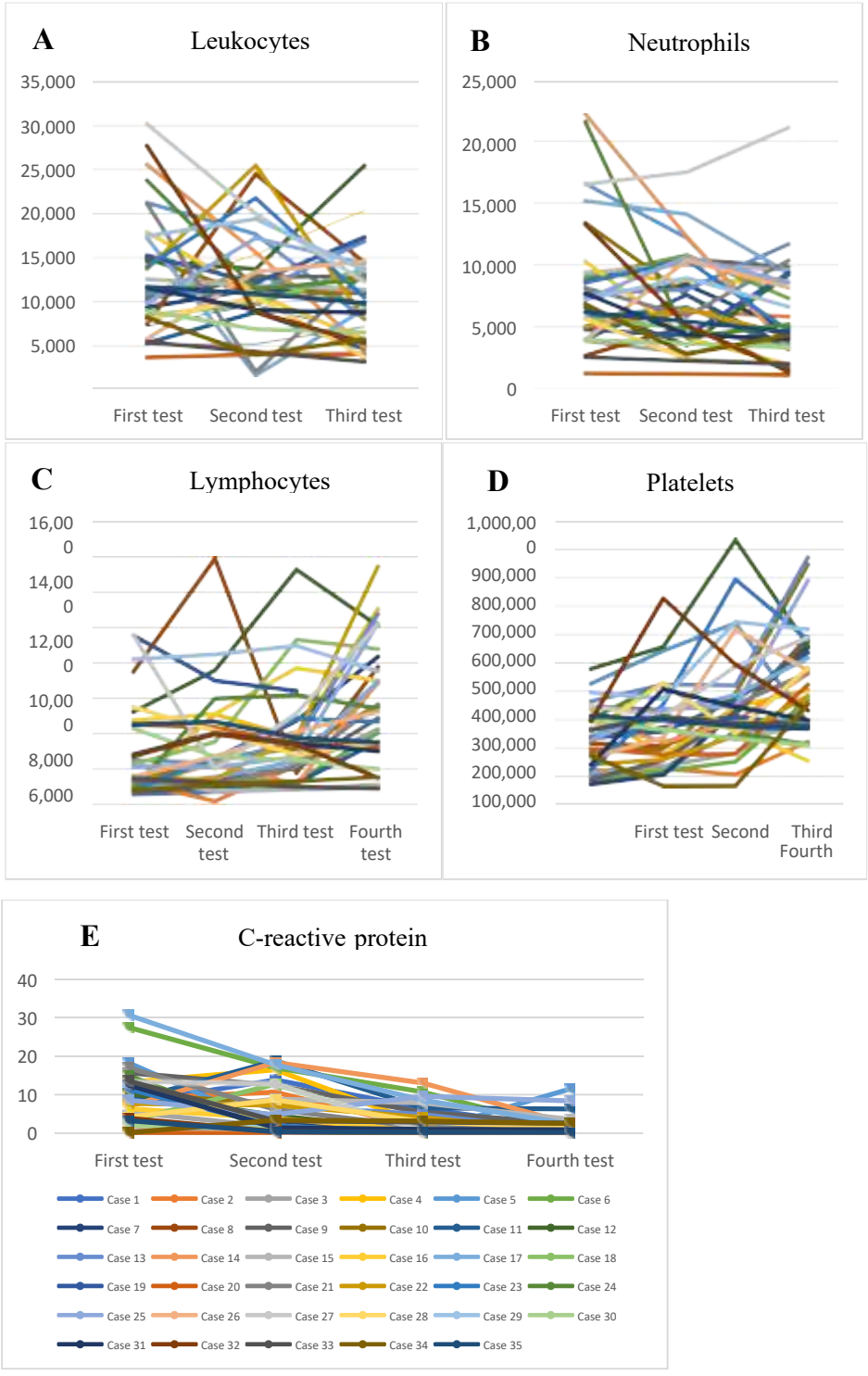


Figure 1. (**A**). The trendline of the values of leukocytes. (**B**). The trendline of the values of neutrophils. (**C**). The trendline of the values of lymphocytes. (**D**). The trendline of the values of platelets. (**E**). The trendline of the values of C-reactive protein.

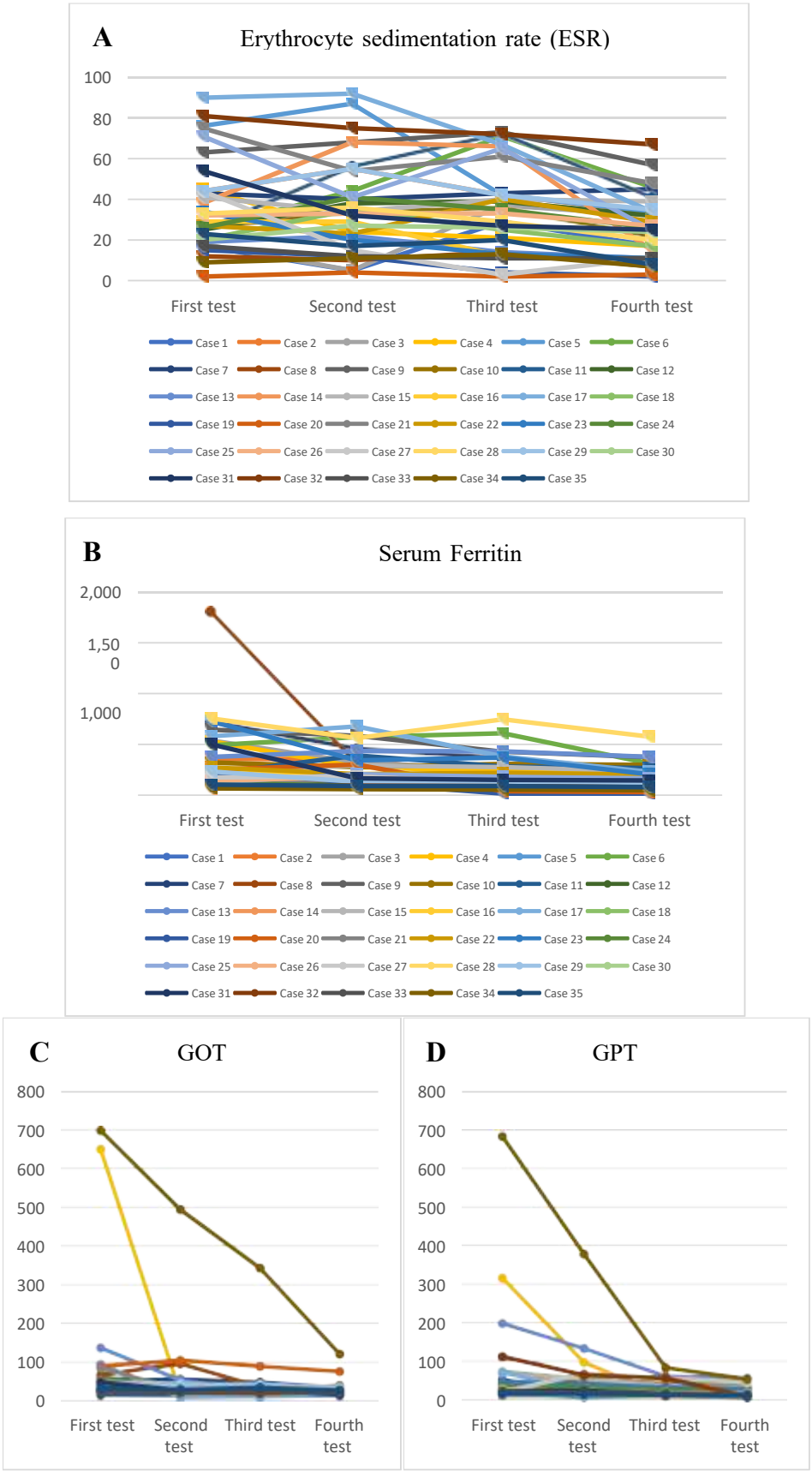


Figure 2. (**A**). The trendline of the values of the erythrocyte sedimentation rate. (**B**). The trendline of the values of serum ferritin. (**C**). The trendline of the values of GOT. (**D**). The trendline of the valuesof GPT.

The activation of immunity caused by SARS-CoV-2 infection leads to excessive cy- tokine production, which is referred to as a "cytokine storm." Increased IL-6 levels were reported in eight patients as a result of this process.

During the hospitalization, two of the patients had involvement of the renal system,as shown by an elevated creatinine level. In addition, a proteinuria was detected during the urinalysis of 34.28% of the cases, and hyperalbuminemia was observed in 17 patients (48.57%). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes were typically increased in 63% of cases (Figure 2C,D), with GOT and GPTlevels over 70 U/l. ($p = 0.01$), in parallel with the elevation of the inflammatory markers and the D-dimer, progressively decreasing after a median of 5 days of treatment, as well as high triglyceride values (Figure 3B).

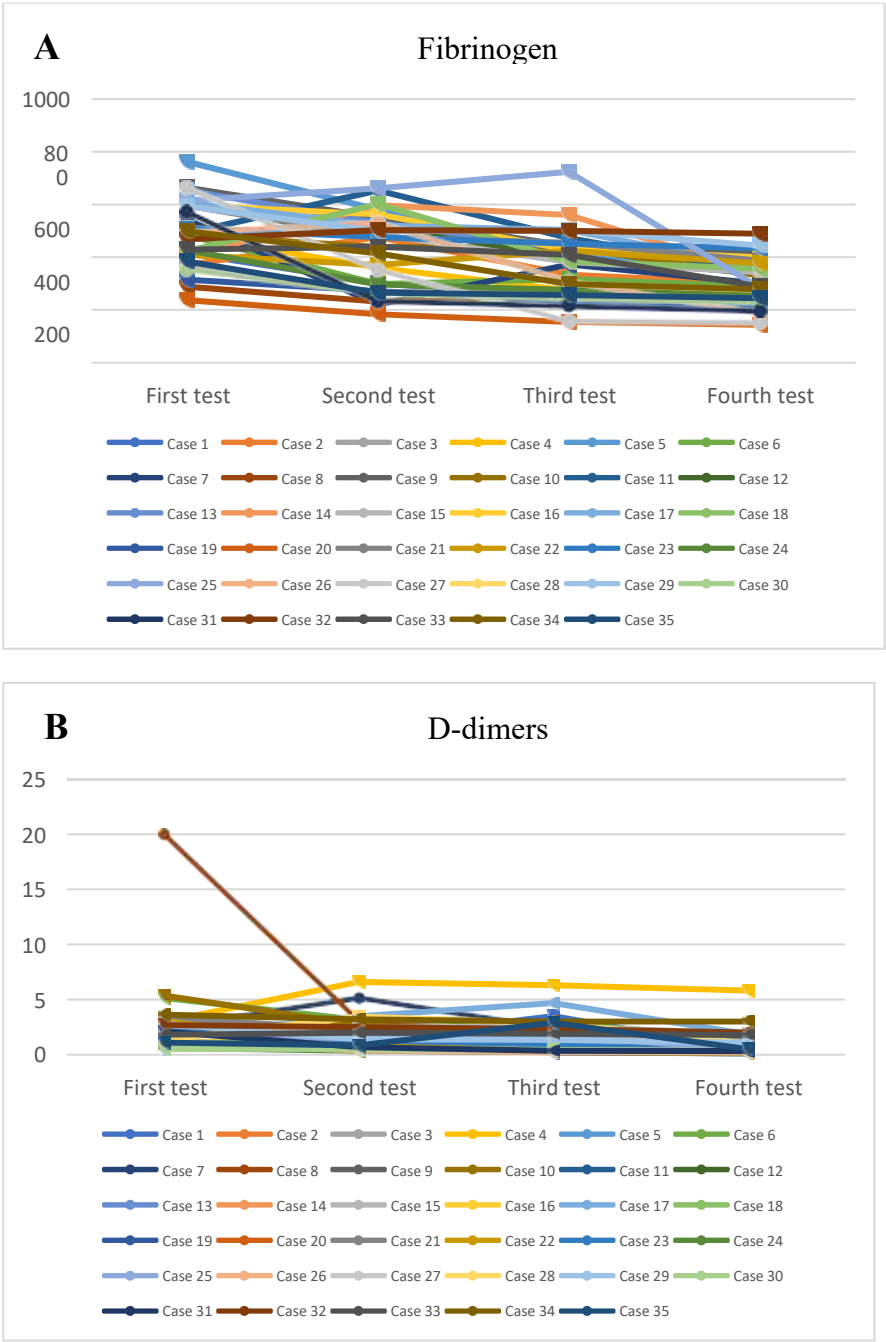


Figure 3. *Cont.*

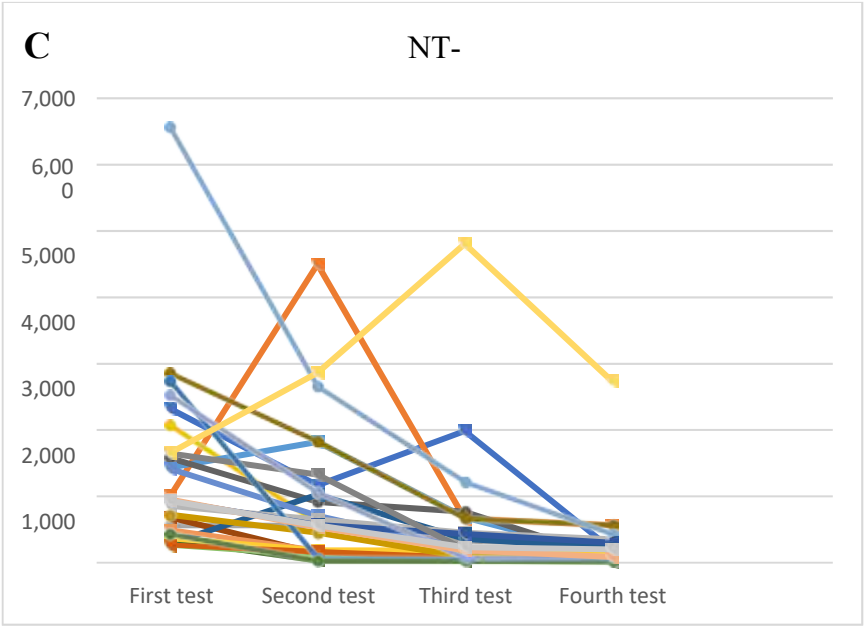


Figure 3. (**A**). The trendline of the values of fibrinogen. (**B**). The trendline of the values of D-dimers.(**C**). The trendline of the values of NT-proBNP.

For the evidence of the prothrombotic status, it was determined that 77.14% of cases (27 patients) presented modified values of fibrinogen ($p = 0.03$, median value 530,000 mg/dL) (Figure 3A), activated partial thromboplastin time (aPTT) ($p = 0.02$, median value 37.9 s),and prothrombin time (PT) ($p = 0.02$) during hospitalization.

Furthermore, significantly high values of N-terminal pro-brain natriuretic peptide (NT-proBNP) were also recorded ($p < 0.001$) (Figure 3C), suggesting cardiac impairment within MIS-C. It was observed that 29 patients (82.85%) had elevated troponin T values ($p = 0.02$), and 28 patients (80%) had increased NT-proBNP values (median 378000 pg/mL). The most common cardiac manifestation was myocarditis, which was present in 43.2% of cases.

After five days in the hospital, cardiac marker values achieved their highest point; further treatment resulted in a gradual improvement, culminating in a normalization of both the appearance of the electrocardiogram and heart function. During the hospital- ization, the patients were treated with intravenous immunoglobulins (IVIG) at a dose of 2 g/kg, cortisone, aspirin, and antibiotics. The patients had a good clinical evolution while they were receiving therapy, which resulted in clinical improvement. Additionally, the inflammatory tests were reduced, with more rapidly normalizing values of C-reactive protein compared with the erythrocyte sedimentation rate and fibrinogen.

In addition, there was a rapid improvement in cardiac enzyme levels, as well as resolution of the fever and rash that had been clinically observed. The patients did not express any negative reactions to the therapy, and it was generally well-received by the patients. A total of 10 days was the typical duration of stay for patients in the hospital (range 7–21 days). Figure 4 presents an illustration of the processes and mechanisms associated with “cytokine storm,” as well as the findings of laboratory experiments. However, the need for long-term monitoring must be taken into consideration. It is still debatable whether a large dosage of acetylsalicylic acid should be used for the therapy, although doing so is essential owing to the significant risk of coronary artery dilatation in one-third of patients who have MIS-C, as shown in recent research.

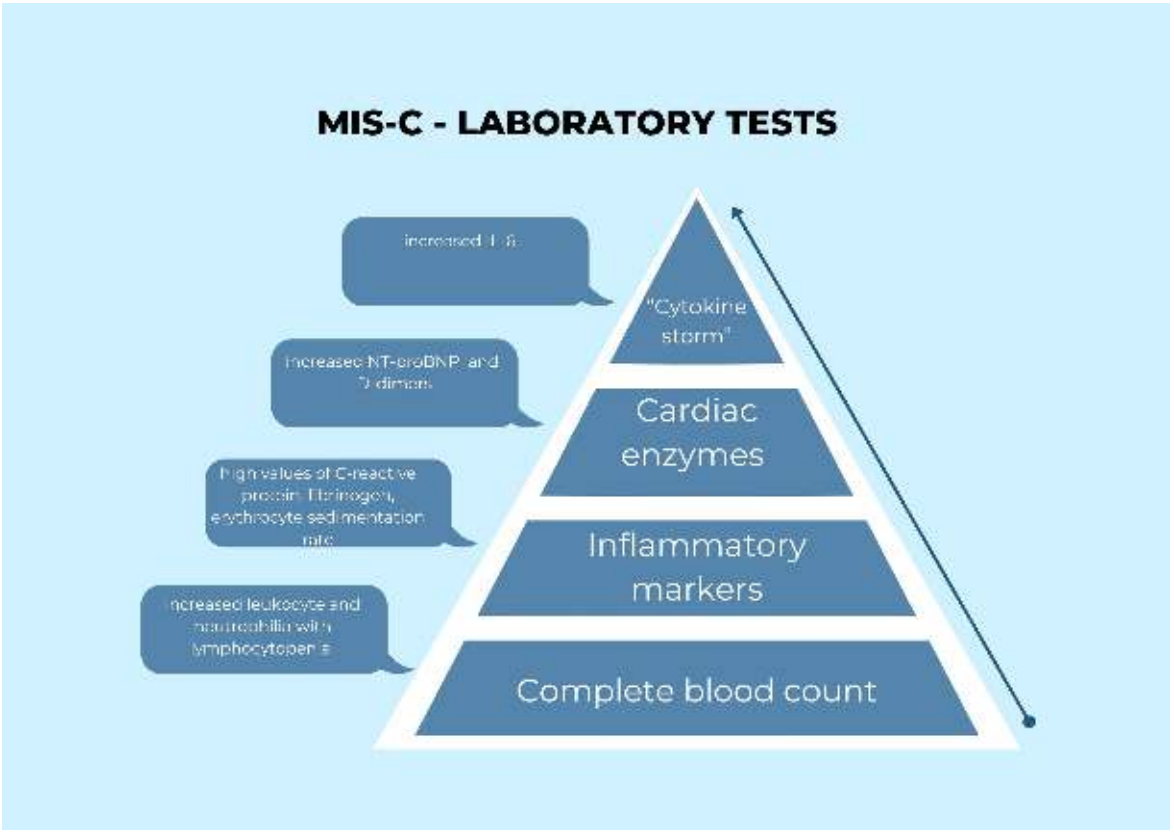


Figure 4. The image presented an illustration of the processes and mechanisms associated with"cytokine storm," as well as the findings of laboratory experiments.

4. Discussion

In addition to cardiac indicators, a complete blood count, an examination of liver enzymes and renal function, as well as an assessment of inflammatory marker levels are all required to be carried out as part of the preliminary laboratory investigations. The majority of patients with MIS-C seem to have an inflammatory state, as shown by neutrophilic leukocytosis, increased erythrocyte sedimentation rates, the decrease in natrium levels, high triglyceride levels, and high reactive-C protein, procalcitonin, d-dimer, and serum ferritin levels.

Laboratory markers of inflammation represent the primary characteristics, which appear to correlate with the severity of multisystem inflammatory syndrome. This is suggested by increased levels of C-reactive protein, fibrinogen, D-dimer, and serum ferritin, as well as decreased values of serum albumin.

High levels of NT-pro-BNP and troponin were detected in our investigation, as well as other studies taken from the published scientific literature [12–15]. These results imply that cardiac dysfunction is a frequent complication associated with MIS-C. Whittaker et al.discovered that 83% of patients had raised NT-pro-BNP levels, whereas 68% had ele- vated troponin levels [14]. Echocardiography in two dimensions must be performed to diagnose myocarditis, pericarditis, valvular anomalies, and coronary artery abnormalities (CAAs) [16–18]. Variations in heart function may sometimes be revealed by electrocar- diograms. Using cardiac magnetic resonance imaging, Blondiaux et al. examined four patients with MIS-C who also had myocarditis. The researchers found that all of the pa- tients had diffuse myocardial edema, which suggests that myocarditis in MIS-C is causedby an infection.

In MIS-C, immunological responses that have not entirely suppressed a continuing infection may make it possible for the intrinsic immune system to stay engaged continu-

ously. This is due to the fact that MIS-C is a type of chronic infection. This pervasive innateimmune inflammatory response is most likely caused by SARS-CoV-2's capacity to inhibittype 1 and type 3 interferon response signaling to the adaptive immune system without impairing cytokine production.

According to the findings of our research, 77.14 percent of the patients had altered values of fibrinogen levels, activated partial thromboplastin time, and prothrombin time, all of which were strongly indicative of a prothrombotic condition ($p = 0.02$). Furthermore, Capone et al. [19] observed 33 patients with MIS-C who presented with high markers of inflammation, particularly fibrinogen and D-dimer concentrations, but no signs of clinicalthrombosis. Although D-dimers have been shown to have elevated fibrinogen and D-dimer concentrations in children with infections and autoimmune disorders such as SARS-CoV-2 and MIS-C, it is possible that D-dimers may not have the specificity necessary for predicting deep venous thrombosis.

In addition to this, the prevalence of thromboembolic consequences in children diag-nosed with MIS-C is undetermined, and there are currently no data-supported guidelines for pediatric thromboprophylaxis. In certain instances, thrombosis has been linked to coagulopathy, despite the fact that the precise mechanism responsible for this condition has not been identified. It is quite uncommon to detect significantly high levels of troponin and NT-pro-BNP in persons who have cardiac involvement, which suggests that myocardial involvement is present.

Despite the well-documented prothrombotic state and thrombotic occurrences in adults infected with SARS-CoV-2, thromboembolic comorbidities in children have not been shown to be similar [20]. In spite of the fact that the precise reason for MIS-C is unknown, researchers have hypothesized that it is caused by an abnormal immune response, which then causes the production of cytokines and organ inflammation [21]. In this regard, MIS-C has been compared to Kawasaki disease, as well as other autoimmune illnesses, all of which have been associated with a thrombogenic condition [22,23]. This comparison is based on the fact that MIS-C is thought to be an autoimmune disease.

Our findings indicate that patients with MIS-C have a hyperinflammatory state, as evidenced by increases in white blood cells, D-dimer, ferritin, and C-reactive protein, as well as fibrinogen, activated partial thromboplastin time, and prothrombin time, all of which are correlated to a hypercoagulable state [24]. It was shown that the INR levels in46.2% of patients were between 1.5 and 2.5 and that the PT was prolonged in 62.1% of cases,even though there were no clinical symptoms of coagulation abnormalities in either group.There have been cases of fulminant myocarditis diagnosed in children that have been reported [25–27]. Polymerase chain reaction testing revealed that these children were infected with SARS-CoV-2. These patients did not suffer from any other serious health conditions. It has been discovered that neonates might develop cardiac abnormalities as early as nine days of life [28].

Myocarditis is a complication that affects between fifty and seventy percent of indi-viduals who develop MIS-C [29,30]. Son et al. found that 47% of 518 pediatric patients diagnosed with MIS-C needed vasopressor therapy, 41% showed depressed left ventricular systolic dysfunction, 12% had coronary artery aneurysm, and 3% needed extracorporeal membrane oxygenation [31]. Additionally, in a case study including 20 patients with MIS-C with cardiac dysfunction, 50% of patients exhibited reduced left ventricular activity and myocardial edema characteristic of myocarditis on cardiac magnetic resonance imaging [32]. Pouletty et al. found that severe myocarditis needed intensive care for almost fifty percent of patients diagnosed with MIS-C [12], with the likelihood of developing the condition increasing with increasing age.

It has been observed that 9–24% of individuals diagnosed with MIS-C had anomalies inthe coronary arteries [9,12–15,33]. The coronary artery anomalies that impact the majority of patients manifest as either a dilatation or a small aneurysm. There have also been reports ofpericarditis, pericardial effusion, and valvular regurgitation [12–15]. Electrocardiographic anomalies include prolonged PR intervals, variations to the T wave, and alterations to theST segment.

In the majority of the studies, almost all patients completely recovered at two weeks,and there is some indication that diastolic dysfunction might remain in a small fraction of individuals six months following acute illness [19]. According to these serious results, the American Academy of Pediatrics recommends that individuals with a confirmation of MIS-C with cardiac dysfunction avoid physical activity for at least 3–6 months post-infection and that permission be acquired from a cardiologist [34].

Myocarditis may rapidly progress; thus, a diagnosis has to be made as soon as possible. In most cases, immunomodulatory therapy is a successful treatment. After a follow-up period of six weeks, an echocardiogram on the patients indicated that the great majority of them had normal activity in the left ventricle [12,13].

As part of the cytokine storm, liver involvement was observed during the MIS-C evaluation. This was accompanied by increased levels of markers for hepatocellular injury (GOT, GPT), low values of serum albumin, and higher IL-6 levels with decreased platelet values. Additionally, the presence of elevated D-dimer values was found to correlate with the liver involvement. According to the findings of Trapani and colleagues, up to 45 percent of children diagnosed with MIS-C might show signs of mild to severe liver damage [35].

Even though the cause of MIS-C may include a number of different factors, further research is necessary to determine the effects that the inflammation has on the heart andwhether or not other organ systems, such as the kidneys and the liver, are also affected.

5. Limitations

Various limitations apply to our investigation. The study was conducted on retrospec- tive data collected from a limited number of pediatric patients, and treatment was adjusted to each person rather than following a defined approach. Second, due to the absence of molecular analysis, our results cannot be used to make true interpretive inferences or address the fundamental pathways of MIS-C. The wide age range of patients (9 months to 15 years) and the relatively small number of children with MIS-C are considered another limitation of this research. This is a pilot study.

6. Conclusions

Patients with clinical manifestations of MIS-C had a negative result on the RT-PCR test for SARS-CoV-2 but had positive levels for IgG antibodies, which is an indicator of previous infection, 4–6 weeks prior to admission.

The pro-inflammatory status was evidenced by elevated levels of C-reactive protein, ESR, fibrinogen, and IL-6. This characteristic of the pro-inflammatory status, as well as multisystem impairment, is highly suggestive of the post-infection immunological reaction of multisystem syndrome, which is temporally associated with SARS-CoV-2 infection.

The clinical characteristics at the onset of the diseases included fever, mucocutaneous manifestations, rash, conjunctivitis, edema of the hands/feet, and strawberry tongue, andmyocardial failure, heart rhythm disturbances, shock, gastro-intestinal manifestations, pul-monary indications, and lymphadenopathy are among the clinical characteristics of MIS-C.The main therapeutic objective was to manage the inflammation process. Due to the cardiovascular involvement, patients needed antiplatelet agents, initially at a high dose,to reduce the incidence of cardiovascular events. Patients need to be monitored for futurecoronary artery dilatation, which, in some cases, may occur, despite an improvement in clinical symptoms and laboratory inflammatory tests.

Children with MIS-C require hospitalization, and some require treatment in a pediatric intensive care unit. Supportive care and treatment must be used to minimize inflammation in damaged organs and prevent them from developing permanent impairments. The long-term follow-up of patients is essential to understanding the long-term implicationsand prognosis for MIS-C patients.

The data that have been presented illustrate the most important laboratory findingsand clinical characteristics of children who have multisystem inflammatory syndrome. These characteristics include the possible involvement of the heart, as well as injury to the liver and an effect on the renal system. This information is useful for the clinical practice of pediatricians. The post-infection immunological reaction of the syndrome that is temporally associated with SARS-CoV-2 infection in children will be appropriately evaluated after the discharge for the follow-up of long-term implications on cardiac, liver,and renal function. The multisystem impairment and pro-inflammatory status are highlysuggestive for the syndrome.

Author Contributions: Conceptualization, C.M.M.; Data curation, A.L.B.; Formal analysis, T.C., C.E.F., L.M. and A.C.P.; Funding acquisition, A.A.; Investigation, C.M.M., T.C., A.L.B. and A.A.; Methodology, C.M.M. and A.C.P.; Project administration, A.A.; Resources, C.M.M., T.C. and C.E.F.; Software, C.E.F.; Supervision, L.M., A.Z.S. and A.A.; Validation, A.Z.S., A.L.B. and A.C.P.; Visualiza-tion, A.A.; Writing—original draft, A.A. and A.Z.S.; Writing—review & editing, A.Z.S. and A.A. Allauthors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Sf Apostol Andrei Clinical County Hospital of Constanta (protocol code no.13/ 11 April 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kurz, H.; Gombala, T. Multisystem Inflammatory Syndrome in Children (MIS-C)—A Case Series in December 2020 in Vienna,Austria. *Front. Pediatr.* **2021**, *9*, 656768. [CrossRef] [PubMed]
2. World Health Organization. Multisystem Inflammatory Syndrome in Children and Adolescents Temporally Related to COVID-19. Scientific Brief, 15 May 2020. WHO: Geneva, Switzerland, 2020. Available online: https://www.who.int/newsroom/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19 (accessed on 1 March 2022).
3. Gruber, C.N.; Patel, R.S.; Trachtman, R.; Lepow, L.; Amanat, F.; Krammer, F.; Wilson, K.M.; Onel, K.; Geanon, D.; Tuballes, K.; et al. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). *Cell* **2020**, *183*, 982–995.e14. [CrossRef]
4. Mazer, M.B.; Bulut, Y.; Brodsky, N.N.; Lam, F.W.; Sturgill, J.L.; Miles, S.M.; Shein, S.L.; Carroll, C.L.; Remy, K.E. Multisystem Inflammatory Syndrome in Children: Host Immunologic Responses. *Pediatr. Crit. Care Med.* **2022**. [CrossRef]
5. Bohn, M.K.; Yousef, P.; Steele, S.; Sepiashvili, L.; Adeli, K. MultiInflammatory Syndrome in Children: A View into Immune Pathogenesis from a Laboratory Perspective. *J. Appl. Lab. Med.* **2021**, *7*, 311–321. [CrossRef]
6. Yonker, L.M.; Gilboa, T.; Ogata, A.F.; Senussi, Y.; Lazarovits, R.; Boribong, B.P.; Bartsch, Y.C.; Loiselle, M.; Rivas, M.N.; Porritt, R.A.; et al. Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier. *J. Clin. Investig.* **2021**, *131*. [CrossRef] [PubMed]
7. Prabhu, S.D. Cytokine-Induced Modulation of Cardiac Function. *Circ. Res.* **2004**, *95*, 1140–1153. [CrossRef] [PubMed]
8. Paediatric Multisystem Inflammatory Syndrome Temporally Associated with COVID-19 (PIMS)-Guidance for Clinicians. Avail- able online: https://www.rcpch.ac.uk/resources/paediatric-multisystem-inflammatory-syndrome-temporally-associated-covid- 19-pims-guidance (accessed on 1 March 2022).
9. Toubiana, J.; Poirault, C.; Corsia, A.; Bajolle, F.; Fourgeaud, J.; Angoulvant, F.; Debray, A.; Basmaci, R.; Salvador, E.; Biscardi, S.; et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: Prospective observational study. *BMJ* **2020**, *369*, m2094. [CrossRef]
10. Penner, J.; Abdel-Mannan, O.; Grant, K.; Maillard, S.; Kucera, F.; Hassell, J.; Eyre, M.; Berger, Z.; Hacohen, Y.; Moshal, K.; et al. 6-month multidisciplinary follow-up and outcomes of patients with paediatric inflammatory multisystem syndrome (PIMS-TS) at a UK tertiary paediatric hospital: A retrospective cohort study. *Lancet Child Adolesc. Health* **2021**, *5*, 473–482. [CrossRef]
11. Bozkurt, B.; Kamat, I.; Hotez, P.J. Myocarditis With COVID-19 mRNA Vaccines. *Circulation* **2021**, *144*, 471–484. [CrossRef]

12. Pouletty, M.; Borocco, C.; Ouldali, N.; Caseris, M.; Basmaci, R.; Lachaume, N.; Bensaid, P.; Pichard, S.; Kouider, H.; Morelle, G.; et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasakidisease (Kawa-COVID-19): A multicentre cohort. *Ann. Rheum. Dis.* **2020**, *79*, 999–1006. [CrossRef]
13. Feldstein, L.R.; Tenforde, M.W.; Friedman, K.G.; Newhams, M.; Rose, E.B.; Dapul, H.; Soma, V.L.; Maddux, A.B.; Mourani, P.M.; Bowens, C.; et al. Characteristics and Outcomes of US Children and Adolescents with Multisystem Inflammatory Syndrome in Children (MIS-C) Compared with Severe Acute COVID-19. *JAMA* **2021**, *325*, 1074–1087. [CrossRef]
14. Whittaker, E.; Bamford, A.; Kenny, J.; Kaforou, M.; Jones, C.; Shah, P.; Ramnarayan, P.; Fraisse, A.; Miller, O.; Davies, P.; et al. Clinical Characteristics of 58 Children with a Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. *JAMA* **2020**, *324*, 259–269. [CrossRef] [PubMed]
15. Lee, P.Y.; Day-Lewis, M.; Henderson, L.A.; Friedman, K.G.; Lo, J.; Roberts, J.E.; Lo, M.S.; Platt, C.D.; Chou, J.; Hoyt, K.J.; et al. Distinct clinical and immunological features of SARS–CoV-2–induced multisystem inflammatory syndrome in children. *J. Clin.Investig.* **2020**, *130*, 5942–5950. [CrossRef] [PubMed]
16. Pilania, R.K.; Jindal, A.K.; Bhattarai, D.; Naganur, S.H.; Singh, S. Cardiovascular Involvement in Kawasaki Disease Is Much MoreThan Mere Coronary Arteritis. *Front. Pediatr.* **2020**, *8*. [CrossRef] [PubMed]
17. Garbin, M.; Raso, I.; Piersanti, A.; Gianolio, L.; De Silvestri, A.; Calcaterra, V.; Corti, C.G.; Nespoli, L.F.; Santacesaria, S.; Fini, G.; et al. Advanced Echocardiographic Analysis in Medium-Term Follow-Up of Children with Previous Multisystem Inflammatory Syndrome. *Children* **2022**, *9*, 917. [CrossRef] [PubMed]
18. Dusad, S.; Singhal, M.; Pilania, R.K.; Suri, D.; Singh, S. CT Coronary Angiography Studies After a Mean Follow-up of 3.8 Years inChildren with Kawasaki Disease and Spontaneous Defervescence. *Front. Pediatr.* **2020**, *8*, 274. [CrossRef]
19. Capone, C.A.; Subramony, A.; Sweberg, T.; Schneider, J.; Shah, S.; Rubin, L.; Schleien, C.; Northwell Health COVID-19 Research Consortium; Epstein, S.; Johnson, J.C.; et al. Characteristics, Cardiac Involvement, and Outcomes of Multisystem Inflammatory Syndrome of Childhood Associated with severe acute respiratory syndrome coronavirus 2 Infection. *J. Pediatr.* **2020**, *224*, 141–145. [CrossRef]
20. Feldstein, L.R.; Rose, E.B.; Horwitz, S.M.; Collins, J.P.; Newhams, M.M.; Son, M.B.F.; Newburger, J.W.; Kleinman, L.C.; Heidemann, S.M.; Martin, A.A.; et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. *N. Engl. J. Med.* **2020**, *383*, 334–346. [CrossRef] [PubMed]
21. Diorio, C.; Henrickson, S.E.; Vella, L.A.; McNerney, K.O.; Chase, J.M.; Burudpakdee, C.; Lee, J.H.; Jasen, C.; Balamuth, F.; Barrett, D.M.; et al. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS–CoV-2. *J. Clin. Investig.* **2020**, *130*, 5967–5975. [CrossRef]
22. Trapani, S.; Rubino, C.; Lasagni, D.; Pegoraro, F.; Resti, M.; Simonini, G.; Indolfi, G. Thromboembolic complications in children with COVID-19 and MIS-C: A narrative review. *Front. Pediatr.* **2022**, *10*. [CrossRef]
23. Hightower, S.; Ellis, H.; Collen, J.; Ellis, J.; Grasso, I.; Roswarski, J.; Cap, A.P.; Chung, K.; Prescher, L.; Kavanaugh, M.; et al. Correlation of indirect markers of hypercoagulability with thromboelastography in severe coronavirus 2019. *Thromb. Res.* **2020**, *195*, 69–71. [CrossRef]
24. Al-Ghafry, M.; Aygun, B.; Appiah-Kubi, A.; Vlachos, A.; Ostovar, G.; Capone, C.; Sweberg, T.; Palumbo, N.; Goenka, P.; Wolfe, L.W.; et al. Are children with SARS-CoV-2 infection at high risk for thrombosis? Viscoelastic testing and coagulation profiles in a case series of pediatric patients. *Pediatr. Blood Cancer* **2020**, *67*, e28737. [CrossRef] [PubMed]
25. Lara, D.; Young, T.; Del Toro, K.; Chan, V.; Ianiro, C.; Hunt, K.; Kleinmahon, J. Acute Fulminant Myocarditis in a Pediatric PatientWith COVID-19 Infection. *Pediatrics* **2020**, *146*, e20201509. [CrossRef] [PubMed]
26. Trogen, B.; Gonzalez, F.J.; Shust, G.F. COVID-19-Associated Myocarditis in an Adolescent. *Pediatr. Infect. Dis. J.* **2020**, *39*, e204–e205. [CrossRef] [PubMed]
27. Gnecchi, M.; Moretti, F.; Bassi, E.M.; Leonardi, S.; Totaro, R.; Perotti, L.; Zuccaro, V.; Perlini, S.; Preda, L.; Baldanti, F.; et al. Myocarditis in a 16-year-old boy positive for SARS-CoV-2. *Lancet* **2020**, *395*, e116. [CrossRef] [PubMed]
28. Hopkins, K.A.; Webster, G. Aberrated supraventricular tachycardia associated with neonatal fever and COVID-19 infection. *BMJ Case Rep.* **2021**, *14*, e241846. [CrossRef] [PubMed]
29. Dufort, E.M.; Koumans, E.H.; Chow, E.; Rosenthal, E.M.; Muse, A.; Rowlands, J.; Barranco, M.A.; Maxted, A.M.; Rosenberg, E.S.; Easton, D.; et al. Multisystem Inflammatory Syndrome in Children in New York State. *N. Engl. J. Med.* **2020**, *383*, 347–358. [CrossRef]
30. Belot, A.; Antona, D.; Renolleau, S.; Javouhey, E.; Hentgen, V.; Angoulvant, F.; Delacourt, C.; Iriart, X.; Ovaert, C.; Bader-Meunier, B.; et al. SARS-CoV-2-related paediatric inflammatory multisystem syndrome, an epidemiological study, France, 1 March to 17 May 2020. *Eurosurveillance* **2020**, *25*, 2001010. [CrossRef]
31. Son, M.B.F.; Murray, N.; Friedman, K.; Young, C.C.; Newhams, M.M.; Feldstein, L.R.; Loftis, L.L.; Tarquinio, K.M.; Singh, A.R.; Heidemann, S.M.; et al. Multisystem Inflammatory Syndrome in Children—Initial Therapy and Outcomes. *N. Engl. J. Med.* **2021**, *385*, 23–34. [CrossRef]
32. Theocharis, P.; Wong, J.; Pushparajah, K.; Mathur, S.K.; Simpson, J.M.; Pascall, E.; Cleary, A.; Stewart, K.; Adhvaryu, K.; Savis, A.; et al. Multimodality cardiac evaluation in children and young adults with multisystem inflammation associated withCOVID-19. *Eur. Heart J. Cardiovasc. Imaging* **2020**, *22*, 896–903. [CrossRef]

33. Ramcharan, T.; Nolan, O.; Lai, C.Y.; Prabhu, N.; Krishnamurthy, R.; Richter, A.G.; Jyothish, D.; Kanthimathinathan, H.K.; Welch, S.B.; Hackett, S.; et al. Paediatric Inflammatory Multisystem Syndrome: Temporally Associated with SARS-CoV-2 (PIMS- TS): Cardiac Features, Management and Short-Term Outcomes at a UK Tertiary Paediatric Hospital. *Pediatr. Cardiol.* **2020**, *41*, 1391–1401. [CrossRef] [PubMed]
34. Risha, K. AAP Updates Guidance on When Youths with Covid-19 Can Return to Physical Activity. 2021. Available online: https://publications.aap.org/aapnews/news/7110?autologincheck=redirected (accessed on 1 March 2022).
35. Pegoraro, F.; Trapani, S.; Indolfi, G. Gastrointestinal, hepatic and pancreatic manifestations of COVID-19 in children. *Clin. Res.Hepatol. Gastroenterol.* **2021**, *46*, 101818. [CrossRef] [PubMed]

medicina

MDPI

Article

Fetal Growth Restriction and Clinical Parameters of Newborns from HIV-Infected Romanian Women

Simona Claudia Cambrea [1,2,†], Elena Dumea [1,2,*], Lucian Cristian Petcu [3], Cristina Maria Mihai [1,†], Constantin Ghita [1,4], Loredana Pazara [1], Diana Badiu [1,*], Costin Ionescu [1], Mara Andreea Cambrea [1], Eosefina Gina Botnariu [5] and Florentina Dumitrescu [6]

1 Faculty of Medicine, "Ovidius" University from Constanta, 900470 Constanta, Romania
2 Clinical Hospital of Infectious Diseases Constanta, 900709 Constanta, Romania
3 Faculty of Dentistry, "Ovidius" University from Constanta, 900470 Constanta, Romania
4 "Dr. Alexandru Gafencu" Emergency Military Hospital, Constanta, 900527 Constanta, Romania
5 Medical Department II, University of Medicine and Pharmacy, "Gr. T. Popa" Iasi, 700115 Iasi, Romania
6 Department of Infectious Disease, Faculty of Medicine, University of Medicine and Pharmacy of Craiova,200349 Craiova, Romania
* Correspondence: elenadumea@yahoo.com (E.D.); dianabadiu@yahoo.com (D.B.)
† These authors contributed equally to this work.

Abstract: *Background and Objectives:* The present study assessed the fetal growth restriction and clinical parameters of both human immunodeficiency virus (HIV)-negative and HIV-positive newborns from HIV-infected mothers in two HIV-acquired immunodeficiency syndrome regional centers (RCs) in Constanta and Craiova, Romania, in order to evaluate the adverse birth-related outcomes. *Materials and Methods:* These represent a retrospective study conducted between 2008 and 2019, in which 408 pregnant HIV-positive women, 244 from Constanta RC and 164 from Craiova RC, were eligible to participate in the study. Consecutive singleton pregnancies delivered beyond 24 weeks of pregnancy were included. Growth restriction in newborns was defined as the birth weight (BW) being less than the third percentile, or three out of the following: BW < 10th percentile; head circumference (HC) < 10th percentile; birth length (BL) < 10th percentile; prenatal diagnosis of fetal growth restriction; and maternal pregnancy information. Of the 244 newborns delivered in Constanta, RC, 17 were HIV-positive, while in Craiova, RC, of the 164 newborns, 9 were HIV-positive. All HIV-positive women were on combined antiretroviral therapy (cART) during pregnancy, similar to all HIV-positive newborns who received ARTs for the first six weeks. We search for the influence of anthropometrical parameters (i.e., HC, BL, and BW), as well as clinical parameters (i.e., newborn sex and Apgar score) for both HIV-negative and HIV-positive newborns, along with the survival rate of HIV-positive newborns. *Results:* There were no differences in the sex of the newborns within either group, with more than 50% being boys. Similarly, the Apgar score did not show any statistically significant values between the two groups (i.e., p = 0.544 for HIV-positive newborns vs. p = 0.108 for HIV-negative newborns). Interestingly, our results showed that in Craiova, RC, there was a chance of 2.16 to find an HIV-negative newborn with an HC < 10th percentile and a 2.54 chance to find an HIV-negative newborn with a BL < 10th percentile compared to Constanta, RC, without any significant differences. On the contrary, Constanta, RC, represented a higher risk of death (i.e., 3.049 times, p = 0.0470) for HIV-positive newborns compared to Craiova, RC. *Conclusions:* Our results support the idea that follow-up of fetal growth restriction should be part of postnatal care in this high-risk population to improve adverse birth-related outcomes.

Keywords: HIV-positive newborns; anthropometrical parameters; fetal growth restriction; Apgarscore; fetal length; birth-related outcome

Citation: Cambrea, S.C.; Dumea, E.; Petcu, L.C.; Mihai, C.M.; Ghita, C.; Pazara, L.; Badiu, D.; Ionescu, C.; Cambrea, M.A.; Botnariu, E.G.; et al. Fetal Growth Restriction and Clinical Parameters of Newborns from HIV-Infected Romanian Women. *Medicina* **2023**, *59*, 111. https://doi.org/10.3390/medicina59010111

Academic Editor: Carlo Torti
Received: 24 December 2022
Revised: 1 January 2023
Accepted: 3 January 2023
Published: 5 January 2023

1. Introduction

Starting in 1990, Romania showed an important cohort of the human immunodeficiency virus (HIV)-infected newborns and children, through unsafe parenteral treatments with reusable unsterilized syringes or blood products [1].

In 2021, approximately 38.4 million people worldwide were living with HIV-infection. In Romania, the number of people undergoing combined antiretroviral therapy (cART) and prophylaxis in September 2022 was 13,985, of which 559 new cases of women were detected in the same year [2]. By using diagnostic tools and proper treatment, the prevention of mother-to-child transmission (MTCT) of HIV-infection can be remarkable.

Nowadays, investigating the fetal growth prospective has increased the attention in detecting different newborns' abnormalities. Understanding the trajectory of fetal growth has become important to prevent birth-related outcomes. It was found that HIV- infection affects more women in comparison to the rest of the population, who tend to seek medical attention at the later stages of the disease as compared to men [3], evenunder the cART prescription [4]. However, it becomes fundamental to know the effects of HIV-infection on pregnancy or offspring anthropometry, with MTCT representing the main consequences, which also increase the risk of adverse birth-related measures. However, attention must be directed to the potential risk for fetal growth restriction along with other important clinical parameters [5]. Although the relationship between intrauterine exposure of fetuses to HIV-infection and the use of cART remains controversial, some studies have suggested cART as a main risk factor. Therefore, if growth restriction results from a direct effect of the virus, immunosuppression, or associated co-morbidities, it is still under debate [6,7]. The study of Beune and contributors gives the final consensus definition of growth restriction in newborns: birth weight (BW) less than the third percentile, or three out of the following: BW < 10th percentile; head circumference (HC) < 10th percentile; birth length (BL) < 10th percentile; prenatal diagnosis of fetal growth restriction; and maternal pregnancy information [8].

Keeping in mind the social or behavioral risk features related to HIV-infection, growth restriction in newborns and clinical parameters may be shared by the same women, result- ing in a high prevalence of adverse newborn outcomes [9].

In the present study, we assessed the anthropometric parameters and clinical features of both HIV-negative and HIV-positive newborns along with the survival rate of HIV- positive newborns from HIV-infected mothers from two HIV-acquired immunodeficiencysyndrome (AIDS) Regional Centers (RCs) Constanta and Craiova from Romania in order to evaluate the adverse birth-related outcomes.

2. Materials and Methods

We conducted a retrospective study on newborns from HIV-positive mothers in two HIV-AIDS RCs from Romania in terms of monitoring HIV-AIDS infection to assess the incidence of fetal growth restriction and clinical parameters among newborns.

During the study period, between 2008 and 2019, 408 HIV-positive pregnant women were monitored, of whom 244 mothers were from HIV-AIDS Constanta, RC, and 164 motherswere from HIV-AIDS Craiova, RC. The population study consisted of participants > 18 years, with a singleton pregnancy. Exclusion criteria included pre-existing hypertension, diabetes, renal, autoimmune, active opportunistic infection for HIV-positive women, morbid obesity, or drug use.

All HIV-positive women were on cARTs (p.o. administration) during pregnancy and childbirth, being administered cARTs, which consisted of triple therapy based on a combination of nucleotide reverse-transcriptase inhibitors, associated with non-nucleoside reverse-transcriptase inhibitors, protease inhibitors, or integrase inhibitors. The mothers' HIV infection was established using the national protocol (e.g., 2 serological enzyme- linked immunosorbent assay (ELISA) positive tests, followed by a Western Blot and HIV- ribonucleic acid (RNA)). According to the prevention of MTCT, for all known HIV-positive women it was a planned Caesarian section (C-section) delivery, but emergency C-section

delivery was recommended in case of pregnancy complications or newly diagnosed HIV pregnant women's status who give birth in the hospital. We included pregnant women known as HIV-infected and those who tested HIV-positive before or during labor.

We studied infants from both centers for 18 months, to establish their HIV status, receiving the standard of care from a dedicated physician according to the Romanian National HIV Management Guidelines. The infant's final HIV status was established after a period of 18 months of follow-up using international recommendations with molecular and serological tests. HIV-RNA determination was performed using COBAS Amplicor TaqMan version 2-0, Roche Molecular Systems Bucharest, Romania (detection limit: 20 copies/mL), and serological tests: ELISA and Western Blot. Newborns received antiretroviral pro- phylaxis for six weeks. The cARTs used in newborns were zidovudine and lamivudine with/without nevirapine or lopinavir/ritonavir. If, at any point in the follow-up period, the newborn's HIV viral load was detectable, we considered an HIV-positive newborn. If during the follow-up period all serological tests remain negative and HIV viral load is undetectable, the newborn was considered HIV-negative [10].

We search the anthropometrical parameters (i.e., HC, BL, and BW) for both HIV- negative and HIV-positive newborns, as well as the clinical parameters (i.e., newborn sex and Agar score), along with the survival rate of HIV-positive newborns. We documented the Apgar score by evaluating five parameters: the color, the heart rate, reflexes, muscle tone, and respiration [11]. Neonatal dimensions were measured within 24 h of birth by neonatal doctors or nurses [12]. In fetal death cases, the autopsy dimensions were used.

Consecutive singleton pregnancies delivered beyond 24 weeks of pregnancy were included and confirmed by an ultrasound examination. Growth restriction in newborns was defined as BW < 10th percentile [8].

The statistical analysis was performed using IBM SPSS statistics software version 23. (Armonk, NY, USA: IBM Corp). The procedures used were descriptive statistics, graphs, and non-parametric statistical tests. Data are presented as medians and IQRs (interquartile range) for continuous variables in cases of skewed distributions or as percentages for categorical variables. For hypotheses testing: Independent Samples Mann Whitney U test, Independent Samples Median test, Chi-Square Test of association, and Chi-squared test for the comparison of two proportions were used depending on the type of analyzed variables. Kaplan-Meier survival analysis was performed using MedCalc statistics software version 14.8.1. (MedCalc Software bvba, Ostend, Belgium, 2014). The significance level (α) was set at 0.05.

Informed consent was obtained from all participants in the study as well as the Agreement of the Ethics Commission (No. 33/22 June 2022) for the publication of the data.

3. Results

3.1. HIV Transmissibility Rates to Newborns

During this period, 244 newborns from Constanta, RC, and 164 newborns from Craiova, RCs, were monitored.

Of the 244 newborns studied in Constanta, RC, 227 were HIV-negative and 17 were HIV-positive, which represented a transmissibility rate of 6.97%, while in Craiova, RC, among 164 newborns from HIV-positive women, 155 were HIV-negative and 9 were HIV-positive, which represented a transmissibility rate of 5.4% (Figure 1). There was no statistically significant difference between the mentioned proportions (Chi-squared = 0.155, df = 1, $p = 0.6941$).

3.2. Clinical and Anthropometrical Parameters of HIV-Positive Newborns

From 26 HIV-positive newborns, 14 (53.84%) were boys (8 from Constanta RC and 6 from Craiova RC) and 12 (46.15%) were girls (9 from Constanta RC and 3 from Craiova RC), with a similar rate in both RCs, without any statistical significance ($p = 0.340$).

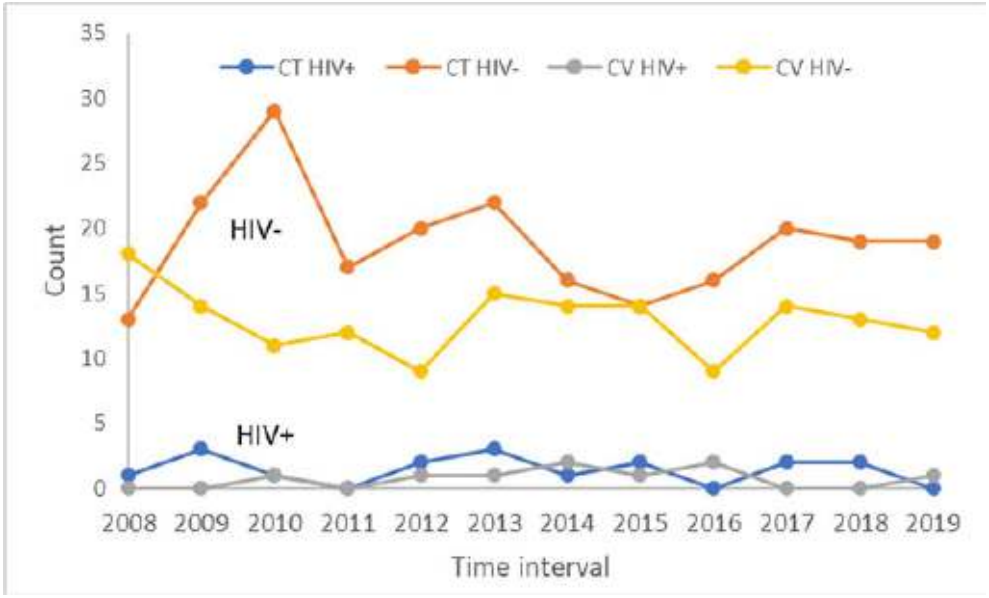


Figure 1. Line chart showing comparative trends over time for HIV-negative and HIV-positive newborns in Constanta and Craiova, Regional Centers (RCs). CT HIV+ = HIV-positive newborns from Constanta RC; CT HIV− = HIV-negative newborns from Constanta RC; CV HIV+ = HIV-positive newborns from Craiova RC; CV HIV− = HIV-negative newborns from Craiova RC.

The Apgar score in the case of HIV-positive newborns performed at birth was between 6 and 10, with a median value of 8 (IQR = 1) for Constanta RC and between 7 and 10, with a median value of 8 (IQR = 1) for Craiova RC, but without any statistical significance between the two groups ($p = 0.544$, Table 1).

Table 1. Anthropometric parameters and Apgar score of HIV-positive newborns.

Infant Parameters	Regional Cente	Minim	Maxim	Median	IQR	*p*
HC (cm)	Constanta	23.00	35.00	32.00	5.00	0.430
	Craiova	26.00	35.00	33.00	4.25	
BL (cm)	Constanta	38.00	51.00	48.00	5.00	0.786
	Craiova	37.00	50.00	48.00	3.50	
BW (g)	Constanta	1500.00	3500.00	2800.00	975.00	0.914
	Craiova	1100.00	3900.00	2640.00	1085.00	
Apgar Score	Constanta	6.00	10.00	8.00	1.00	0.544
	Craiova	7.00	10.00	8.00	1.00	

HC = head circumference; BL = birth length; BW = birth weight; IQR = interquartile range (75th percentile (P75)–25th percentile (P25)).

Table 1 presents the anthropological parameters and the Apgar score for HIV-positive newborns from the two RCs.

The test does not reveal significant differences between the HC HIV-positive newborns from Constanta RC (Median = 32, $n = 17$) and the HC HIV-positive newborns from Craiova, RC (Median = 33, $n = 9$), $U = 62.00$, $z = 0.789$, $p = 0.430$.

There was no association between the variables in each RC and the newborns' HC < 10th percentile (i.e., $\chi^2_{stat} = 0.026$, df = 1, $p = 0.873 > \alpha = 0.05$). The chance of finding a newborn with HC < 10th percentile present in the group of patients from Constanta RC is equal to

the chance of finding a newborn with HC < 10th percentile present in the group of patients from Craiova, RC: OR = 1.143; 95% CI for OR = (0.224, 5.841) (Figure 2).

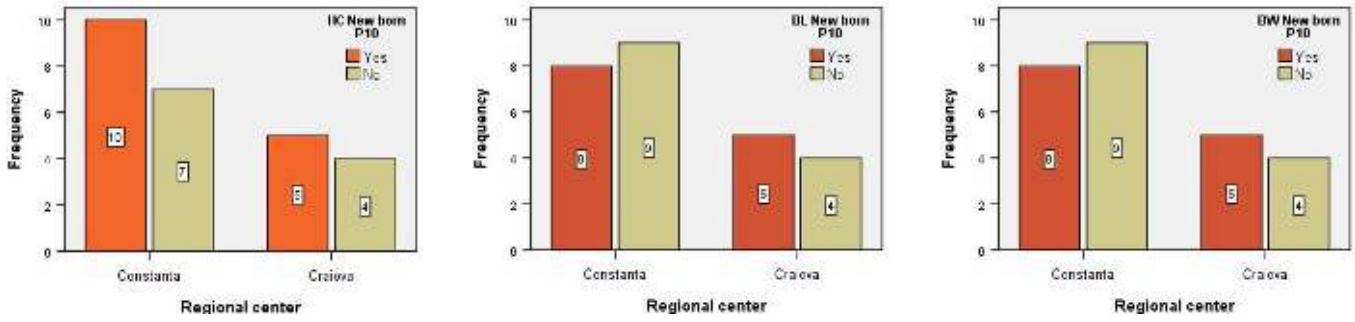


Figure 2. Anthropometric parameters (**a**) head circumference (HC), (**b**) birth length (BL), and (**c**) birthweight (BW) of HIV-positive newborns from the two Regional Centers. P10 = percentile 10.

The test does not reveal significant differences between the BL HIV-positive newborns from Constanta, RC (Median = 48, n = 17), and the BL HIV-positive newborns from Craiova, RC (Median = 48, n = 9), U = 71.50, z = 0.272, p = 0.786.

There was no association between the variables in each RC and BL HIV-positive newborns with < 10th percentile (i.e., χ^2_{stat} = 0.170, df = 1, p = 0.680 > α = 0.05). The chance of finding a newborn with BL < 10th percentile present in the group of patients from Constanta, RC, is equal to the chance of finding a newborn with BL < 10th percentile present in the group of patients from Craiova, RC: OR = 0.711; 95% CI for OR = (0.140, 3.606)(Figure 2).

The test does not reveal significant differences between the BW HIV-positive newborns from Constanta, RC (Median = 2800.00, n = 17), and the BW HIV-positive newborns from Craiova, RC (Median = 2640.00, n = 9), U = 74.50, z = 0.108, p = 0.914.

There was no association between the variables of each RC and the BW HIV-positive newborns (i.e., χ^2_{stat} = 0.170, df = 1, p = 0.680 > α = 0.05). The chance of finding a newborn with BW < 10th percentile present in the group of patients from Constanta, RC, is equal to the chance of finding a patient with BW < 10th percentile present in the group of patients from Craiova, RC: OR = 0.711; 95% CI for OR = (0.140, 3.606) (Figure 2).

3.3. Clinical and Anthropometrical Parameters of HIV-Negative Newborns

From 382 HIV-negative newborns, 194 (50.78%) were boys (121 from Constanta, RC, and 73 from Craiova, RC), and 188 (49.21%) were girls (106 from Constanta, RC, and 82 from Craiova, RC), with a similar rate in both RCs but without any statistical significance(p = 0.233).

The Apgar score in the case of HIV-negative newborns performed at birth, was between 4 and 10, with a median value of 9 (IQR = 1) for Constanta, RC, and between 5 and 9, with a median value of 9 (IQR = 1) for Craiova, RC, without any statistical significance between the two groups (p = 0.108, Table 2).

Table 2 presents the anthropological parameters and the Apgar score for HIV-negative newborns from the two RCs.

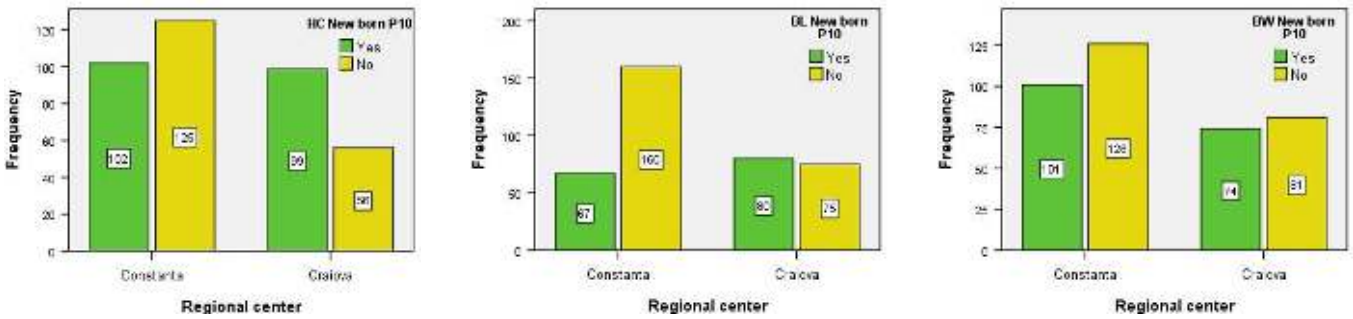
The test does not reveal significant differences between the HC HIV-negative newborns from Constanta, RC (Median = 33, n = 227), and the HC HIV-negative newborns from Craiova, RC (Median = 33, n = 155), U = 16693.5, z = −0.861, p = 0.389.

Table 2. Anthropometric parameters and Apgar score of HIV-negative newborns.

Infant Parameters	Regional Cente	Minim	Maxim	Median	IQR	*p*
HC (cm)	Constanta	23.00	38.00	33.00	3.00	0.389
	Craiova	24.00	37.00	33.00	2.00	
BL (cm)	Constanta	35.00	55.00	48.00	4.00	0.758
	Craiova	35.00	52.00	48.00	3.00	
BW (g)	Constanta	1000.00	4000.00	2800.00	700.00	0.221
	Craiova	950.00	4300.00	2830.00	720.00	
Apgar Score	Constanta	4.00	10.00	9.00	1.00	0.108
	Craiova	5.00	9.00	9.00	1.00	

HC = head circumference; BL = birth length; BW = birth weight, IQR = interquartile range (75th percentile (P75)–25th percentile (P25)).

There was an association between the variables in each RC and the HC HIV-negativenewborns (i.e., $\chi^2_{stat} = 13.249$, df = 1, $p < 0.001 < \alpha = 0.05$). The chance of finding a newborn with the HC < 10th percentile present in the group of patients from Constanta, RC, is 2.16 (1/0.462) times lower than the chance of finding a patient with the HC < 10thpercentile present in the group of patients from Craiova, RC: OR = 0.462; 95% CI forOR = (0.303, 0.702) (Figure 3).



(**a**) (**b**) (**c**)

Figure 3. Anthropometric parameters (**a**) head circumference (HC), (**b**) birth length (BL), and (**c**) birthweight (BW) of HIV-negative newborns from the two Reginal Centers. P10 = percentile 10.

The test does not reveal significant differences between the BL HIV-negative newborns from Constanta, RC (Median = 48.00, *n* = 227), and BL HIV-negative newborns from Craiova, RC (Median = 48, *n* = 155), *U* = 17269, z = 0.308, *p* = 0.758.

There was an association between the variables in each RC and the BL HIV-negativenewborns (i.e., $\chi^2_{stat} = 18.999$, df = 1, $p < 0.001 < \alpha = 0.05$). The chance of finding a newborn with BL < 10th percentile present in the group of patients from Constanta, RC, is 2.54 (1/0.393) times lower than the chance of finding a newborn with BL < 10th percentile present in the group of patients from Craiova, RC: OR = 0.393; 95% CI for OR = (0.257, 0.600)(Figure 3).

The test does not reveal significant differences between the BW HIV-negative newbornsfrom Constanta, RC (Median = 2800.00, *n* = 227), and the BW HIV-negative newborns from Craiova, RC (Median = 2830, *n* = 155), *U* = 16296.5, z = 1.224, *p* = 0.221.

There was no association between the variables in each RC and the BW HIV-negativenewborns (i.e., $\chi^2_{stat} = 0.392$, df = 1, $p = 0.531 > \alpha = 0.05$). The chance of finding a newborn with BW < 10th percentile present in the group of patients from Constanta, RC, is equal tothe chance of finding a newborn with BW < 10th percentile present in the group of patients from Craiova, RC: OR = 0.877; 95% CI for OR = (0.582, 1.322) (Figure 3).

3.4. Survival of HIV-Positive Newborns

From the 408 newborns from the HIV-positive women analyzed in the two RCs,9 infants (3.69%) from the 17 HIV-positive from Constanta, RC, and 2 infants (1.22%) from the 9 HIV-positive from Craiova, RC, reached the final event, death (Figure 4).

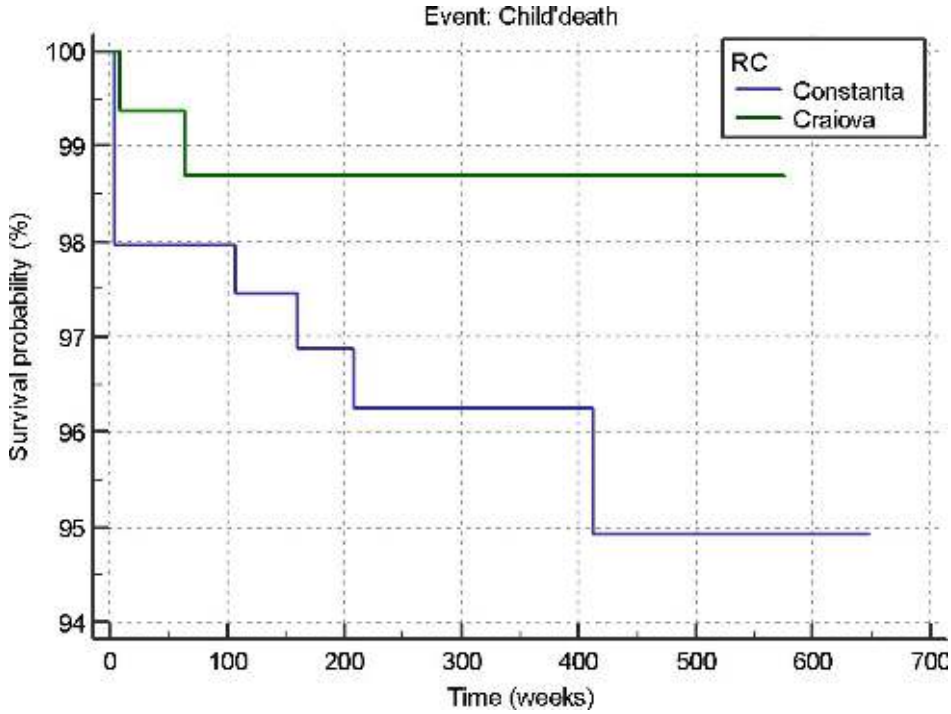


Figure 4. Survival curve of HIV-positive newborns for each RC.

The two survival curves differ significantly: Chi-square = 3.945, df = 1, $p = 0.0470 < 0.05$. Therefore, the estimated risk of death in the case of newborns from Constanta, RC, was 3.049 times higher than that in Craiova, RC (the risk ratio HR = 3.042 and the 95% CI forHR = (1.0844 to 10.3381)).

4. Discussion

The staff at our clinic continues to improve the healthcare provided to HIV-infectedpatients, especially the women of childbearing age, to allow them the chance to have a healthy child [13–18]. Our study shows that there were no differences in the sex of the newborns between the Constanta and Craiova RCs, with more than 50% being boys. Similarly, the Apgar score didn't show any statistically significant values between the two groups (i.e., $p = 0.544$ for HIV-positive infants vs. $p = 0.108$ for HIV-negative infants).

There was no dependent relationship between the variables in each RC and fetal growth restriction (i.e., $\chi^2_{stat} = 0.454$, df = 1, $p = 0.500 > \alpha = 0.05$) in the HIV-positive newborns. The chance of finding a newborn with growth restriction present in the groupof patients from Constanta, RC, was equal to the chance of finding a newborn with growth
restriction present in the group of patients from Craiova, RC: OR = 1778; 95% CI forOR = (0.331, 9.554).

There was no dependent relationship between the variables in each RC and fetal growth restriction (i.e., $\chi^2_{stat} = 2.213$, df = 1, $p = 0.137 > \alpha = 0.05$) in the HIV-negative newborns. The chance of finding a newborn with growth restriction present in the groupof patients from Constanta, RC, was equal to the chance of finding a newborn with growth
restriction present in the group of patients from Craiova, RC: OR = 0.704; 95% CI for OR(0.443, 1.119).

Therefore, in Craiova, RC, there was a 2.16 percent chance of finding a newborn withan HC < 10th percentile compared to Constanta, RC. Similarly, in the same region, there

was also a 2.54 chance of finding a newborn with a BL < 10th percentile compared to theConstanta, RC, being at higher risk of fetal growth restriction.

However, the Constanta RC represented a higher risk of death (i.e., 3.049 times, $p = 0.0470$) among HIV-positive newborns compared to the Craiova RC.

If, in the case of anthropometric parameters (i.e., HC and BL), Craiova RC presented a higher risk compared to Constanta RC, and in the case of the estimated risk of death, Constanta RC presented a higher risk compared to Craiova RC. The effect of HIV-infection risk factors on adverse perinatal outcomes has not yet been disclosed. Risk factors such as poverty, lack of social support, anemia, diabetes, hypertension, chemo- or radiotherapy including different oncologic treatments [19], bariatric surgery involvement [20], or other pathogenic agents similar to hyper-virulent *Klebsiella pneumoniae* [21] could have important involvement on growth restriction, preterm birth, or even mortality of newborns [22].

The most dangerous consequences for newborns occur when the mother is primarily infected in the first trimester of pregnancy. Although a vaccine is not actually available to avoid HIV-primary infection, prevention through hygienic measures represents the only way to prevent infections. These mainly include avoiding contact with children and washing hands thoroughly after this kind of contact [23].

Therefore, it is difficult to notice the independent feature of each risk factor in women with HIV-infection and to be able further to make clear preventions [24].

However, some studies showed low BW or premature children born from HIV-infected mothers [25–27]. We did not, however, find a significantly increased risk of low BW in women with HIV-infection in the present study.

Delicio and contributors showed high rates of neonatal adverse outcomes on 793 preg- nancies from HIV-positive mothers. About 22.5% of the children had low BW, 22% wereborn prematurely, 18% were SGA, and 4% had very low BW [28]. Another study had similar results, with 74 children exposed to maternal cART between 2001 and 2012, in which 34.8% were exposed since conception, preterm birth rates of 17.5%, and low BW of 20.2%, with higher rates of HIV-infected mothers [9].

In the case of cART exposure, it showed a higher incidence of prematurity or low BW in children. Moreover, C-section was linked with a higher occurrence in low BW, being used especially in chronic distress or low fetal supply. National guidelines from 23 European countries recommended that HIV-positive women on successful cART with a very low orundetectable viral load < 1000 can have a vaginal delivery [29].

In one of our previous studies from the Constanta RC, the mean BW was 2670 g, and the percentage of BW < 10th percentile was 58.05%. Infants who presented < 10th percentilefor weight and length were 22.76%. About 11.38% of infants were < 10th percentile for weight, length, and cranial circumference [30]. However, our results did not show any association between the two RCs, with different materno-fetal monitoring possibilities and the presence of fetal growth restriction.

5. Conclusions

HIV-positive women who received cART did not appear to have newborns with abnormal fetal growth, as evidenced by no significant difference in anthropometrical or clinical parameters between both HIV-positive and HIV-negative newborns. Finally, our results support the notion that follow-up of fetal growth restriction should be part of postnatal care in this high-risk population to improve adverse birth-related outcomes.

Author Contributions: S.C.C. and C.M.M.—substantial contribution to the conception of the work, conceived and designed the study, supervision and final approval of the version to be published; D.B. and E.D.—substantial contribution in collection data, conceived and designed the study and final approval of the version to be published; L.C.P.—substantial contribution in performing statistical analysis, and final approval of the version to be published; C.G., M.A.C., L.P., C.I. and E.G.B.— conceived and designed the study, revising the paper critically for important intellectual content, final approval of the version to be published; F.D. data curation, design the study and methodology.All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki as well as the Agreement of the Ethics Commission of both Clinical Hospitals of Infectious Diseases from Constanta RC (No.33/22 June 2022) and Craiova RC (No.8507/14 June 2022) for the publication of the data.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data of this report are available from the corresponding authors upon request.

Acknowledgments: We appreciate the effort of the personnel from the Day Clinic from Constanta RC and Craiova RC for medical and psycho-social support and for helping us to collect data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. The Romanian Academy 'Stefan S. Nicolau' Institute of Virology. Available online: https://www.virology.ro/en/research/hiv-aids/highlights.html (accessed on 28 October 2022).
2. Department for Monitoring and Evaluation of HIV/AIDS Infection in Romania, National Institute of Infectious Diseases 'Prof.Dr.Matei Bals'. Available online: https://www.cnlas.ro/images/doc/01122022.pdf (accessed on 12 October 2022).
3. Padyana, M.; Bhat, R.V.; Dinesha; Nawaz, A. HIV in Females: A Clinico-epidemiological Study. *J. Fam. Med. Prim. Care* **2013**, *2*, 149–152. [CrossRef]
4. Deeks, S.; Tracy, R.; Douek, D. Systemic effects of inflammation on health during chronic HIV infection. *Immunity* **2013**, *39*, 633–645. [CrossRef]
5. Ørbæk, M.; Thorsteinsson, K.; Moseholm Larsen, E.; Katzenstein, T.; Storgaard, M.; Johansen, I.; Pedersen, G.; Bach, D.; Helleberg, M.; Weis, N.; et al. Risk factors during pregnancy and birth-related complications in HIV-positive versus HIV-negative women in Denmark, 2002–2014. *HIV Med.* **2020**, *21*, 84–95. [CrossRef] [PubMed]
6. Cotter, A.M.; Garcia, A.G.; Duthely, M.L.; Luke, B.; O'Sullivan, M.J. Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth? *J. Infect. Dis.* **2006**, *193*, 1195–1201. [CrossRef] [PubMed]
7. Fleiss, B.; Wong, F.; Brownfoot, F.; Shearer, I.K.; Baud, O.; Walker, D.W.; Gressens, P.; Tolcos, M. Knowledge Gaps and Emerging Research Areas in Intrauterine Growth Restriction-Associated Brain Injury. *Front. Endocrinol.* **2019**, *10*, 188. [CrossRef]
8. Beune, I.M.; Bloomfield, F.H.; Ganzevoort, W.; Embleton, N.D.; Rozance, P.J.; van Wassenaer-Leemhuis, A.; Wynia, K.; Gordijn, S.J. Consensus Based Definition of Growth Restriction in the Newborn. *J. Pediatr.* **2018**, *196*, 71–76.e1. [CrossRef]
9. Dos Reis, H.L.; Araujo Kda, S.; Ribeiro, L.P.; Da Rocha, D.R.; Rosato, D.P.; Passos, M.R.; Merçon De Vargas, P.R. Preterm birth and fetal growth restriction in HIV-infected Brazilian pregnant women. *Rev. Inst. Med. Trop. Sao Paulo* **2015**, *57*, 111–120. [CrossRef]
10. WHO Recommendations on the Diagnosis of HIV Infection in Infants and Children. Available online: https://www.ncbi.nlm.nih.gov/books/NBK138552/ (accessed on 2 October 2022).
11. The Apgar Score. Committee Opinion No. 644. American College of Obstetricians and Gynecologists. *Obs. Gynecol.* **2015**, *126*, e52–e55. [CrossRef] [PubMed]
12. Hasmasanu, M.G.; Bolboaca, S.D.; Baizat, M.I.; Drugan, T.C.; Zaharie, G.C. Neonatal short-term outcomes in infants with intrauterine growth restriction. *Saudi Med. J.* **2015**, *36*, 947–953. [CrossRef]
13. Cambrea, S.C.; Marcu, E.A.; Cucli, E.; Badiu, D.; Penciu, R.; Petcu, C.L.; Dumea, E.; Halichidis, S.; Pazara, L.; Mihai, C.M.; et al. Clinical and Biological Risk Factors Associated with Increased Mother-to-Child Transmission of HIV in Two South-East HIV-AIDS Regional Centers in Romania. *Medicina* **2022**, *58*, 275. [CrossRef]
14. Cambrea, S.C.; Popescu, G.G.; Resul, G.; Petcu, L.C. The spectrum of infectious diseases hospital mortality by HIV status. *Acta Med. Mediterr.* **2019**, *35*, 3517–3522.
15. Leopa, N.; Dumitru, E.; Dumitru, A.; Tocia, C.; Prazaru, M.D.; Costea, D.O.; Popescu, R.C. The Clinicopathological Differences of Colon Cancer in Young Adults versus Older Adults. *J. Adolesc. Young Adult Oncol.* **2022**. *Epub ahead of print*. [CrossRef] [PubMed]
16. Stefanopol, I.A.; Baroiu, L.; Constantin, G.B.; Danila, D.M.; Anghel, L.; Nechifor, A.; Tatu, A.L. Diagnostic and Management of Undescended Ovary–A Preoperative Dilemma: A Case-Based Systematic Review. *Int. J. Women's Health* **2022**, *14*, 15–27. [CrossRef]
17. Zugravu, C.; Nanu, M.I.; Moldovanu, F.; Arghir, O.C.; Mihai, C.M.; Otelea, M.R.; Cambrea, S.C. The influence of Perinatal Education on Breastfeeding Decision and Duration. *Int. J. Child Health Nutr.* **2018**, *7*, 74–81. [CrossRef]
18. Pătru, S.; Pădureanu, R.; Dumitrescu, F.; Pădureanu, V.; Rădulescu, D.; Dragoi, D.; Matei, D. Influence of multidisciplinary therapeutic approach on fibromyalgia patients. *Exp. Ther. Med.* **2021**, *21*, 528. [CrossRef] [PubMed]
19. Ghita, C.; Vilcea, I.D.; Dumitrescu, M.; Vilcea, A.M.; Mirea, C.S.; Aschie, M.; Vasilescu, F. The prognostic value of immunohis- tochemical aspects of tumor suppressor genes p53, bcl-2, PTEN and nuclear proliferative antigen Ki-67 in resected colorectal carcinoma. *Rom. J. Morphol. Embryol.* **2012**, *53*, 549–556. [PubMed]

20. Bjørklund, G.; Peana, M.; Pivina, L.; Dosa, A.; Aaseth, J.; Semenova, Y.; Chirumbolo, S.; Medici, S.; Dadar, M.; Costea, D.O. Iron Deficiency in Obesity and after Bariatric Surgery. *Biomolecules* **2021**, *11*, 613. [CrossRef] [PubMed]
21. S¸ erban, D.; Popa Cherecheanu, A.; Dascalu, A.M.; Socea, B.; Vancea, G.; Stana, D.; Smarandache, G.C.; Sabau, A.D.; Costea, D.O. Hypervirulent *Klebsiella pneumoniae* Endogenous Endophthalmitis—A Global Emerging Disease. *LIFE-BASEL* **2021**, *11*, 676. [CrossRef]
22. Sebastiani, G.; Borrás-Novell, C.; Casanova, M.A.; Pascual Tutusaus, M.; Ferrero Martínez, S.; Gómez Roig, M.D.; García-Algar, O.The Effects of Alcohol and Drugs of Abuse on Maternal Nutritional Profile during Pregnancy. *Nutrients* **2018**, *10*, 1008. [CrossRef]
23. Mazzitelli, M.; Micieli, M.; Votino, C.; Visconti, F.; Quaresima, P.; Strazzulla, A.; Torti, C.; Zullo, F. Knowledge of Human Cytomegalovirus Infection and Prevention in Pregnant Women: A Baseline, Operational Survey. *Infect. Dis. Obstet. Gynecol.* **2017**, *2017*, 5495927. [CrossRef]
24. Birkhead, G.S.; Pulver, W.P.; Warren, B.L.; Hackel, S.; Rodríguez, D.; Smith, L. Acquiring human immunodeficiency virus during pregnancy and mother-to-child transmission in New York: 2002–2006. *Obstet. Gynecol.* **2010**, *115*, 1247–1255. [CrossRef]
25. Zenebe, A.; Eshetu, B.; Gebremedhin, S. Association between maternal HIV infection and birthweight in a tertiary hospital in southern Ethiopia: Retrospective cohort study. *Ital. J. Pediatr.* **2020**, *46*, 70. [CrossRef]
26. Fentie, E.A.; Yeshita, H.Y.; Bokie, M.M. Low birth weight and associated factors among HIV positive and negative mothers delivered in northwest Amhara region referral hospitals, Ethiopia, 2020 a comparative crossectional study. *PLoS ONE* **2022**, *17*, e0263812. [CrossRef] [PubMed]
27. Xiao, P.L.; Zhou, Y.B.; Chen, Y.; Yang, M.X.; Song, X.X.; Shi, Y.; Jiang, Q.W. Association between maternal HIV infection and lowbirth
28. weight and prematurity: A meta-analysis of cohort studies. *BMC Pregnancy Childbirth* **2015**, *15*, 246. [CrossRef]
29. Delicio, A.M.; Lajos, G.J.; Amaral, E.; Cavichiolli, F.; Polydoro, M.; Milanez, H. Adverse effects in children exposed to maternal HIV and antiretroviral therapy during pregnancy in Brazil: A cohort study. *Reprod. Health* **2018**, *15*, 76. [CrossRef]
30. Kennedy, C.E.; Yeh, P.T.; Pandey, S.; Betran, A.P.; Narasimhan, M. Elective caesarean section for women living with HIV: Asystematic review of risks and benefits. *AIDS* **2017**, *31*, 1579–1591. [CrossRef] [PubMed]
31. Cambrea, S.C.; Tănase, D.E.; Ilie, M.M.; Diaconu, S.; Marcaş, C.; Carp, D.S.; Halichidis, S.; Petcu, L.C. Can HIV infection during pregnancy cause an intrauterine growth restriction? *BMC Infect. Dis* **2013**, *13*, O5. [CrossRef]

Diagnostics

MDPI

Review

Continuous Glucose Monitoring in Transient Neonatal Diabetes Mellitus—2 Case Reports and Literature Review

Tatiana Chisnoiu [1,2], Adriana Luminita Balasa [1,2], Larisia Mihai [1,2], Ancuta Lupu [3,*], Corina Elena Frecus [1,2], Irina Ion [1,2], Antonio Andrusca [1,2], Alexandru Cosmin Pantazi [1,2], Maria Nicolae [1,2], Vasile Valeriu Lupu [3,*], Constantin Ionescu [4], Cristina Maria Mihai [1,2,*] and Simona Claudia Cambrea [5]

[1] Department of Pediatrics, Faculty of General Medicine, "Ovidius" University, 900470 Constanta, Romania
[2] Pediatrics, County Clinical Emergency Hospital of Constanta, 900591 Constanta, Romania
[3] Pediatrics, "Grigore T. Popa", Department of Mother and Child Medicine, University of Medicine and Pharmacy, 700115 Iasi, Romania
[4] Department 1 Preclinical, Faculty of General Medicine, "Ovidius" University, 900470 Constanta, Romania
[5] Department of Infectious Diseases, Faculty of General Medicine, "Ovidius" University, 900470 Constanta, Romania
* Correspondence: anca_ign@yahoo.com (A.L.); valeriulupu@yahoo.com (V.V.L.); cristina2603@yahoo.com (C.M.M.)

Abstract: Neonatal diabetes mellitus is a rare genetic disease that affects 1 in 90,000 live births. The start of the disease is often before the baby is 6 months old, with rare cases of onset between 6 months and 1 year. It is characterized by low or absent insulin levels in the blood, leading to severe hyperglycemia in the patient, which requires temporary insulin therapy in around 50% of cases or permanent insulin therapy in other cases. Two major processes involved in diabetes mellitus are a deformed pancreas with altered insulin-secreting cell development and/or survival or faulty functioning of the existing pancreatic beta cell. We will discuss the cases of two preterm girls with neonatal diabetes mellitus in this research. In addition to reviewing the literature on the topic, we examined the different mutations, patient care, and clinical outcomes both before and after insulin treatment.

Keywords: neonatal diabetes; glucose monitoring; monogenetic diabetes

Citation: Chisnoiu, T.; Balasa, A.L.; Mihai, L.; Lupu, A.; Frecus, C.E.; Ion, I.; Andrusca, A.; Pantazi, A.C.; Nicolae, M.; Lupu, V.V.; et al. Continuous Glucose Monitoring in Transient Neonatal Diabetes Mellitus—2 Case Reports and Literature Review. *Diagnostics* **2023**, *13*, 2271. https://doi.org/10.3390/diagnostics13132271

Academic Editors: Marijn Speeckaert, Liliana-Georgeta Foia, George Alexandru Maftei and Cristiana Elena Vlad

Received: 25 April 2023
Revised: 3 June 2023
Accepted: 30 June 2023
Published: 4 July 2023

1. Introduction

Neonatal diabetes mellitus (NDM), also known as monogenetic diabetes of infancy, is an uncommon condition that most often affects newborns or young children under the age of six months. When a baby at term has intrauterine growth restriction (IUGR) and an early, suboptimal postnatal weight evolution, neonatal diabetes should be considered a differential diagnosis [1].

Currently, there are two categories that are identified based on clinical and genetic factors:

- permanent neonatal diabetes mellitus (PNDM)
- transient neonatal diabetes mellitus (TNDM)

It is estimated that between 1 in 215,000 and 1 in 400,000 newborns have diabetes mellitus [1]. The diabetes in around 50% of these infants is just temporary. According to research, chromosome 6q24 methylation defects (paternal uniparental disomy, paternal duplication, and loss of methylation without a structural defect) account for about 70% of these cases [2]. These conditions are characterized by:

- moderate or severe intrauterine growth restriction
- early development (during the first week of life)
- rare, mild, non-ketotic hyperglycemia

In the southeastern Anatolian area of Turkey, Demirbilek et al. discovered that the total yearly incidence of NDM (including PNDM) is 1 in 30,000 live births (with PNDM 1 in 48,000 live births). In this Turkish cohort, the prevalence of TNDM due to changes in the ZFP57 gene was equally great, with two out of three TNDM patients with anomalies in themethylation of chromosome 6q24 having mutations in the ZFP57 gene. For the reason that there is a 25% chance of recurrence in affected families with such a historical background, TNDM may be suspected. This is due to the occurrence of ZFP57 gene mutations, which correlate with consanguinity [3]. To determine the actual prevalence rate of NDM, further research is required. Given that the pattern of inheritance for the genes involved differs greatly, genetic counseling for NDM patients is dependent on the genetic etiology [4].

Affected men have a 50% probability of transferring TNDM to their descendantsin family situations where there is a paternal duplication of the chromosome 6q24 area. Children will not be impacted by maternal duplication, but the boys may be able to pass on TNDM to their children [5]. Few cases of transient diabetes are linked to functional abnor- malities of KATP channels on the β-cell membrane (activating mutations of the ABCC8 and KCNJ11 genes), but some clinical characteristics, such as higher birth weight, later onset of hyperglycemia, and relatively delayed remission, may still exist [6]. Recently, other genetic subtypes were discovered, including certain transcription factors involved in pancreatic development (GATA6, PAX6, NEUROG3, and NEUROD1), especially those connected to a phenotypic range that includes extrapancreatic characteristics and diabetes [7].

2. Case Presentations

We present two instances of preterm girls who had intrauterine growth restriction (IUGR) (<3rd percentile) and were delivered at 37 weeks and 35 weeks of gestation, respec- tively. The characteristics of both cases are presented in Table 1.

Table 1. Clinical characteristics and laboratory results of the patients.

Patient	**Case Report 1**	**Case Report 2**
Gender	F	F
Age	First day of life	First day of life
Birth weightLength	1480 g—37 weeks of gestation Severe Intrauterine growth restriction IUGR < 3rd percentile 43 cm	2100 g—35 weeks of gestation Intrauterine growth restriction (Oligohydramnios) 44 cm
Head circumference	29 cm	31 cm
Feeding type	Formula-fed up to 13 days of life, then breastfed	Mixed fed—breastfed and formula fed sincethe first day of life
Clinical features	facial dysmorphism epicanthus umbilical hernia	facial dysmorphism epicanthus macroglossia pectus excavatum umbilical hernia short hallux
Glucose level at diagnosis	275 mg/dL	350 mg/dL
Genetic test	Uniparental disomy at the 6q24 locus	Uniparental disomy at the 6q24 locus
Treatment	Insulin Lispro was started continuous with a rhythm between 0.1–0.3 mL/h (0.01–0.03 units/kg/h) for the first days and constantly 0.1 mL/h after that(0.43 units/24 h); At 2 months—insulin pump with a dose 0.05 units/h	Insulin Lispro was started continuous with a rhythm between 0.026 units/kg/day and then reduced gradually up to 0.006 units/kg/day;

Table 1. *Cont.*

Patient	Case Report 1	Case Report 2
Evolution	Discharge after 2 months and 2 weeks Lispro Insulin dose was reduced gradually and subsequently was stopped one week following her discharge; At the age of 12 months, the baby develops very well, normal anthropometric parameters, no infectious episodesand no hyper- or hypoglycemic events.	Discharge at 2 months when treatment with Lispro insulin was stopped; At age of 6 months, the baby develops very well, with normal anthropometric parameters, no infectious episodes and nohyper- or hypoglycemic events.

In the first case, the baby's blood glucose level increased from 275 mg/dL (on thefirst day) to 370 mg/dL. For the first few days, the continuous insulin infusion was initiated at a rate of 0.1–0.3 mL/h (0.01–0.03 units/kg/h), and thereafter it was maintained at 0.1 mL/h continuously (0.43 units/24 h).

In the second case, the baby's blood glucose levels increased from 350 mg/dL on day one to 467 mg/dL on day two. The insulin infusion was begun continuously at a rhythm of 0.026 units/kg/day and subsequently progressively decreased to 0.006 units/kg/day as glucose levels fell between 50 and 240 mg/dL.

Due to the increased risk of hyperglycemia and glycemic fluctuation, we used con- tinuous glucose monitoring in both cases. The insulin rhythm was regularly changed in response to feedings and blood sugar measurements. We used for CGM The FreeStyle Libre system which is authorized for the control of diabetes in children older than 4 years old. When the FreeStyle Libre system is scanned or "flashed" across the sensor, it displaysthe interstitial glucose level. Older versions of the FreeStyle system lacked alerts to warn patients of high or low blood sugar. Another method for transmitting glucose to authorized users is to scan the sensor with a mobile phone equipped with near-field communication. The glucose variance over the last 24 h is shown, and trend arrows are shown. The sensordoesn't require any calibration and runs for 14 days before switching off on its own. It isthe least expensive of the current technologies, with accuracy comparable to that of the Dexcom G6. For use in neonatal critical care, the system lacks a license.

In the first case report, after two months, the patient was switched from Insulin drip to continuous and customized doses of rapid-acting Insulin (Lispro). This was completedthrough an insulin pump whose rhythm was 0.05 units/h for blood glucose levels that varied from 80 to 180 mg/dL. After 2.5 months, the patient was sent home in excellent general health with a weight of 4080 g (Figure 1). With a body weight that exceeded the50th percentile, the second patient likewise made significant progress (Figure 2).

In both instances, the hyperglycemia was caused by a uniparental disomy at the 6q24 locus (caused by paternal disomy). With the assistance of the University of Exeter, Medical School, Diabetes Genes, we carried out this genetic test. DNA samples from patients with methylation loss were discovered using methylation-specific copy number analysis on the 6q24 gene. A microsatellite study of nine polymorphic chromosome 6 markers revealed no maternal contribution. These findings support the diagnosis of temporary neonatal diabetes caused by paternal uniparental disomy at locus 6q24.

In both situations, the infants have normal anthropometric profiles, no infectious episodes, and neither hyper- nor hypoglycemia occurrences. The need for long-term follow-up was explained to the parents since there are no clinical, biochemical, or genetic connections to predict a potential recurrence, and the probability of recurrence is not tobe ignored.

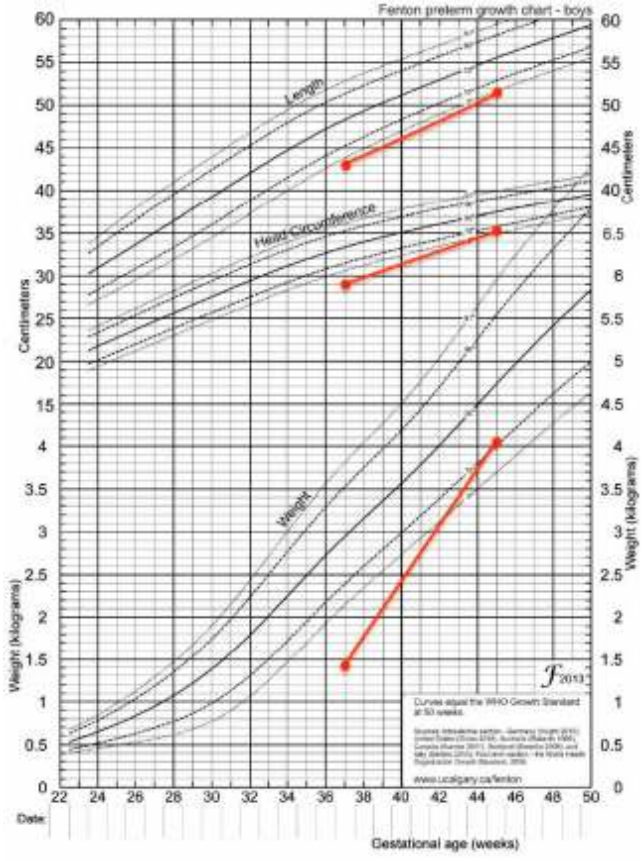


Figure 1. Growth chart-case 1.

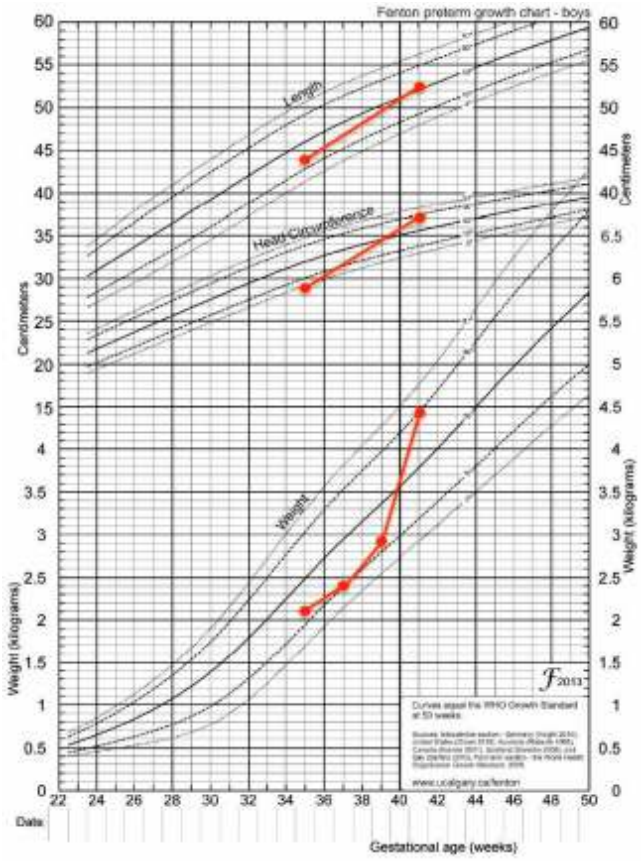


Figure 2. Growth chart-case 2.

3. Discussion

Neonatal diabetes is a rare condition associated with low birth weight due to dis-turbance of insulin secretion in the intrauterine period [7]. In both of our cases, the birth weight was below the 3rd centile. DNA testing is needed to confirm the molecular etiology of NDM. These tests are expensive and not accessible in Romania, but some centers outside Romania offer them at no cost for research purposes.

Some patients with neonatal diabetes can be associated with neurological diseases characterized by developmental delay, muscle weakness, seizures, and dysmorphic fea- tures [8]. These patients present a type of mutation in the KCNJ11 gene. The catch-up growth is usually present in most cases once the insulin treatment starts, with a good prognosis [9]. In our cases, the growth chart showed that the somatometric evolution was very good.

Insulin, irrespective of the etiology, is the cornerstone of the therapy of NDM in the early postnatal period. Oral sulfonylurea, which is taken in accordance with the genetic diagnosis of KATP channel mutations, is another viable therapeutic option. The administration of insulin should begin as soon as diabetes is identified by persistent hyperglycemia [10]. We promptly began insulin treatment in both situations.

In practice, fast-acting insulins cost about the same but are more expensive than the previous animal source and biosynthetic regular human insulin preparations. All three can be used in infants, children, adolescents, adults, and the elderly without realizing that they are ideally administered about 15 min before meals to try and control the glycemic fluctuations of most foods and snacks and that they would need to be coupled with some form of basal/background insulin for glycemic control between meals. All fast-acting insulin analogs share the same potential benefits and have minimal differences in terms of clinical performance, reducing nocturnal and postmeal hypoglycemia, and improving coverage for postmeal glycemic excursions [11].

Transient neonatal diabetes mellitus caused by genetic anomalies of the imprinted locus at 6q24 is known as 6q24-related transient neonatal diabetes mellitus, or 6q24-TNDM. Severe intrauterine growth restriction, hyperglycemia (from the neonatal period and before 18 months), dehydration, and the lack of ketoacidosis are the key characteristics [12].

Deafness, severe hypotonia, congenital cardiac problems, renal malformations, and neurologic characteristics including epilepsy may also be present in 6q24-TNDM linked with multilocus imprinting disease (MLID). Diabetes mellitus often starts during the first week of life and lasts three months on average; however, it may last longer, sometimes for up to a year. Although insulin is often required at first, the requirement for it gradually diminishes. Children may have sporadic periods of hyperglycemia, particularly when they are unwell. In adolescence or even dulthood, diabetes mellitus may return. Pregnant women who have experienced 6q24-TNDM are at risk for a recurrence [13].

Docherty et al. investigated the relationship between genotype and phenotype in a global sample of TNDM patients. According to their findings, umbilical hernia (21%) and macroglossia (44%) were the two most prevalent congenital defects. On the other hand, dysmorphic facial features (18%), renal tract anomalies (double kidneys, hydronephrosis, dilated renal pelvis, and vesicoureteral reflux) (9%), cardiac anomalies (ductus arteriosus, tetralogy of Fallot, atrial septal defects, and persistent foramen ovale) (9%), clinodactyly, polydactyly, nail and short finger anomalies (8%), and hypothyroidism (4%) were among the congenital abnormalities that occurred less frequently [14].

Transient neonatal diabetes is an anomaly of the 6q24 locus involving the ZAC (Z finger protein that regulates apoptosis and cell cycle arrest; also known as PLAGL1–pleo- morphic adenoma gene-like 1) and HYAMI genes [15]. The 6q24 variant is also associated with macroglossia and umbilical hernias. The macroglossia was present only in case 2, and the umbilical hernia was present in both our cases.

A proband with transient neonatal diabetes mellitus and a DNA methylation study revealing relative hypomethylation within the differentially methylated region 6q24 (DMR) is diagnosed with 6q24-TNDM.

Overexpression of the imprinted genes PLAGL1 and HYMAI on 6q24 leads to 6q24- TNDM. These genes' shared promoter contains the DMR (i.e., PLAGL1 TSS alt-DMR). Only the paternal alleles of PLAGL1 and HYMAI are expressed under normal circum- stances because DMR methylation suppresses the expression of the maternal alleles of these genes [16]. Additional molecular genetic testing may reveal the genetic process at play, which is necessary for genetic counseling. Three distinct genetic mechanisms, including paternal uniparental disomy of chromosome 6 [17], duplication of 6q24 on the paternal allele [18], and hypomethylation of maternal PLAGL1 TSS alt-DMR [19], which results in inappropriate expression of the maternal PLAGL1 and HYMAI alleles, cause duplication of the normal dosage of PLAGL1 and HYMAI (causing 6q24-TNDM).

Shield et al.'s cohort analysis of newborns with TNDM revealed that the median age of initiation of insulin treatment was 12 weeks, the majority of patients were small for gestational age, and the average age of presentation was three days after being born [20]. In TNDM, hyperglycemia is strikingly severe and often accompanied by low or undetectable levels of C-peptide and insulin. Although ketoacidosis may only very rarely result from hyperglycemia, it is more probable in PNDM than in TNDM. More than 95% of TNDM patients have IUGR, which commonly appears in the third trimester [21]. According to 2002 French cohort research, TNDM was linked to higher rates of IUGR (74 vs. 36%) and earlier diagnosis (median age, six days; range, 1–81 days versus median age, 27 days; range, 1–127 days) than PNDM. When compared with individuals with K-ATP channel mutations, patients with TNDM who have abnormalities on chromosome 6q had considerably lowerbirth weights [22].

It is impossible to utilize low HbA1c (high HbF) as an indication of glycemic control in NDM because of how it is seen in relation to plasma glucose levels [23,24]. The insulin secretion deficit is similar in type 1 and neonatal diabetes. Neonatal diabetes often begins with ketoacidosis. Ketoacidosis from the onset of neo-natal diabetes is treated in the same way as in the case of type 1 diabetes. The child's life will be saved by insulin treatment, which must be started right away [25].

As neonates are very sensitive to insulin and at risk of developing severe hypo- glycemia, insulin treatment should be started with caution. Regular insulin is continuously infused intravenously at 0.05–0.1 U/kg/h and adjusted depending on the blood glucose levels. Therapy aims to restore fluid and electrolyte balance as well as enable tissues to use normal amounts of energy [26].

For newborns with IUGR in particular, insulin therapy is essential to provide optimal weight gain and development; however, treating NDM is challenging since there is a lack of subcutaneous fat, and the need for low doses of insulin. A continuous subcutaneous insulin infusion (CSII), intermittent subcutaneous treatment, or intravenous in-fusion of insulin are several ways in which we can make sure insulin is provided [27]. After the initial treatment of diabetic ketoacidosis, infants with persistent hyperglycemia, despite decreases in glucose infusion rates, and those with persistent glucose excursions should continue receiving regular insulin intravenously, while others are switched to a suitable subcutaneous insulin schedule. Finding a treatment plan that works is often difficult since there is little information on the best insulin doses for young newborns. Patients are switched over to basal insulin injections or a basal/bolus insulin regimen [28].

A significant problem in NDM continues to be the administration of small insulin doses, fluctuating insulin needs, and frequent blood glucose testing. For neonates with diabetes, CSII allows for modest rates of insulin administration [29]. Furthermore, CSII offers more adaptability to account for the variation in oral intake as well as adjustments in energy expenditure as the kid develops. In contrast to injections, CSII is safer, more physiological, simpler to control, and allows for the administration of relatively little insulin [30].

An electrode sensor used in continuous glucose monitoring systems (CGMS) catalyzes the oxidation of glucose, producing an electric current that is measured by a monitor. The interstitial fluid's glucose level may be continuously measured thanks to the CGMS,

which is placed subcutaneously. The sensor is particularly helpful for premature and small gestational-age infants who are susceptible to significant fluctuations in blood glucose levels [31]. When glucose levels are either lower than acceptable limits or higher than acceptable limits, the CGMS is set to provide alarms, allowing the caregiver to react quickly. Since both hypoglycemia and hyperglycemia are linked to immediate neurophysiological abnormalities and long-term neurodevelopmental impairment, timely treatment is crucial from a therapeutic perspective. However, more research is needed as there is not enough experience with its usage or accuracy in infants, despite its claimed benefits for continuous blood glucose testing [32].

According to two recent investigations, needle sensors were also well tolerated in newborns weighing more than 1.5 kg and had no negative breastfeeding effects [33,34]. These trials included newborns as little as 579 g in weight. There were no reports of any severe symptoms, including infection, edema, hemorrhage, or bruising, and the sensors were utilized for up to 7 days with no apparent performance degradation [35,36]. According to Galderisi et al., sensor detachment was a concern in a small number of extremely preterm newborns. However, in the case of two patients, CGM had to be discontinued due to repeated detachments [37].

The evaluation of neonatal hypoglycemia is severely constrained by the fact that glucose concentrations are only provided in the range of 40 mg/dL (2.2 mmol/L) to 400 mg/dL (22 mmol/L), which is a significant restriction of present technology. Point-to-point recalibration of the raw signal, which also enhances the accuracy of CGM in the lower glucose range, may, however, overcome this for retrospective analysis [38]. The sensors' need for a "wetting" phase, which normally lasts for around 2 h, is another drawback. However, neonates have not been particularly tested to see how long it takes for the signal output to stabilize. According to Harris et al. [39], the mean absolute error peaked on the first day after insertion, which may be partially attributable to increased error during the wetting period.

When converting sensor current to blood glucose concentration, the continuous shift internal algorithm used by CGM is updated by periodic calibration based on blood glucose readings [40]. The three primary types of mistakes that might occur with CGM have some major factors, such as: zero-mean error, drift, and diffusion time delay. Due to the technology used in the sensor and its interstitial location, the zero mean error is the random error of the sensor. The error itself can be rather large. Drift is the term used to describe changes in sensor output between calibration points brought on by biofilm or corrosion on the needle surface, which results in varying currents for the same blood glucose level. While it has not been researched in newborns, the possibility of sensor drift in adults is widely established. A delay is also caused by the diffusion of glucose between the interstitial and vascular compartments. This is around 20 min in lambs [39], which is in line with circumstantial evidence in neonates [34,41,42]. This time delay has the practical effect of making CGM's positive and negative errors become larger when blood glucose levels increase and decrease, respectively.

The incidence and duration of hypoglycemia in type 1 diabetes have been demon- strated to be decreased using a CGM and insulin pump used together with a computer algorithm (artificial pancreas) [43]. A potential treatment strategy for hyperglycemic preterm newborns is computerized insulin dosage based on predicted insulin sensitiv- ity [43,44]. Although it has been reported that using CGM in conjunction with insulin infusion lowers the incidence of hypoglycemia episodes in a newborn with neonatal dia-betes, there is presently no data on the use of CGM to advise insulin therapy in neonatal hyperglycemia [45].

Before CGM may be suggested for real-time monitoring in neonatal intensive care, several obstacles must be resolved. CGM has considerable promise for optimizing blood glucose levels in newborns [40]. In both situations, we successfully employed CGM and saw no negative effects. The last-generation technological innovation, CGM, contributes towards achieving good metabolic control. Up to 67% of all patients with type 1 diabetes

used CGM, according to a study conducted in Germany [44]. Reducing hypoglycemia is an important key that can be resolved by assessing the risk factors for problematic hypoglycemia and by introducing advanced diabetes technologies into the management of diabetes [45].

Once NDM is detected, glycemic management and the avoidance of both hyper- and hypoglycemia are the main therapy objectives. Real-time CGM can be a helpful tool for preserving secure glucose control during insulin treatment [46].

Prolonged or severe hyperglycemia or hypoglycemia can be lessened using CGM. The best glucose goals, how to achieve them, and the possible impact on long-term healthoutcomes will need to be determined by more research utilizing CGM [47].

The future of managing diabetes is undoubtedly CGM. Any young children, includingneonates, babies, and preschoolers, as well as any kids of any age with cognitive or neurodevelopmental issues that affect their capacity to detect or react to hypoglycemia, should be given CGM consideration [48].

The reduction of hypoglycemia, in particular the minimization of severe hypoglycemia, is a goal in the treatment of children and adolescents with type 1 diabetes. Assessing the riskfactors for developing severe hypoglycemia is of great importance in preventing dangerous hypoglycemia from occurring [49]. The National Institute for Clinical Excellence (NICE)now recommends that adults and children with diabetes who are at risk of hypoglycemia should use CGM. Hypoglycemia is common in the neonatal period and a preventable cause of poor neurodevelopmental outcomes. Newborn studies have shown that CGM can detect clinically silent hypoglycemia, which has been associated with reduced executive and visual function in early childhood [49].

All neonates with NDM should be fed a high-calorie diet and given enough insulin to support healthy weight gain and development. The amount of carbohydrates in normal human milk and regular baby formula is comparable (about 70 to 75 g/L), but the amount of carbohydrates in enriched human milk is very small (0.1 g/packet). To address calorie requirements and carbohydrate counts, dietary management includes a team of profession- als that can advise on this matter [6]. We provide all the information available to encourage exclusive breastfeeding and to develop programs that promote a balanced diet for infants throughout the first year of life [50]. Therefore, carbohydrate estimation for breastfed infants can be challenging. If a patient is fed pumped breast milk, the carbohydrate content can be estimated at 2.1 g per ounce of breast milk. Resources are available to help caregivers estimate the amount of breast milk and, subsequently, the carbohydrates consumed [51].

Around 50% of neonatal diabetes cases see a spontaneous remission, often within the first three months (transient diabetes). However, a clinical return is always a possibility. For the remaining 50%, permanent neonatal diabetes requires a lifetime of medication to regulate glycemia [51].

After puberty, TNDM sufferers frequently experience relapses. Relapsed 6q24-related diabetes is often seen in non-obese, autoantibody-negative patients and is no longer a transitory condition [49]. A considerable portion of patients with relapsing diabetes mellitus (DM) after adolescence have been reported to have insulin insufficiency but not obesity [50]. The first instance of relapsing 6q24-TNDM with predominant insulin resistance is reported by Uchida et al. [52].

Many 6q24-TNDM individuals experience recurrent DM after puberty and are born small for gestational age (SGA) [53]. SGA and puberty are well-known risk factors for increasing insulin resistance. According to Docherty LE et al., individuals with 6q24-TNDM had mean (SD) birth weights and gestational weeks of 2001 (417) g and 37.8 (2.7) weeks, respectively [14].

Metz et al. describe in a large cohort of patients with TNDM and PNDM, that five TNDM patients experienced the onset of permanent insulin-dependent diabetes after the age of 8, highlighting the importance of continuing follow-up. The 19 TNDM patients who underwent testing had two paternal isodisomies of chromosome 6, seven paternally- derived trisomies from four families, and two methylation defects in the 6q24 area [22].

Transient neonatal diabetes subtypes 6q24/TNDM and KATP/TNDM have unique clinical characteristics that are caused by various disease processes [54].

In a recent study completed in 2016, Besser et al. included 750 children who had diabetes that had started before they were six months old. The authors said that out of this cohort, 604 patients were born at or before 37 weeks' gestation, whereas the other patients (n = 146) were [55,56].

A number of papers have noted people with 6q24-related diabetes who do not have a history of TNDM [57]. Table 2 summarizes the gene mutations involved in transient neonatal diabetes mellitus.

Table 2. Gene mutations in transient neonatal diabetes mellitus and Therapy [58].

Mechanism of β-Cell Dysfunction	Gene Mutation	Chromosome Locus	Inheritance	Additional Features	Therapy
Reduced β-cell development	*ZAC* (*IPLAG1*)/*HYMA1*	6q24	imprinting; AD	macroglossia; umbilical hernia	insulin
	ZEP57	6p22.1	AR		insulin
	HNF1B	17q21.3		pancreatic cysts hypoplasia; renal	
Failure membrane to depolarize	*KCNJ11* (Kir6.2)	11p15.1	AD; de novo	low developmental birth weight; delay; DEND	sulfonylurea
Failure channel to close KATP	*ABCC8* (*SUR1*)	11p15.1	AD; AR; de novo	low birth weight	sulfonylurea
Abnormal β-cell function	*INS* (proinsulin)	11p15.5	AR	low birth weight	Insulin

Expanded methods of genetic assessment for TNDM have been explored as molecular testing develops. A new study investigated 1020 neonatal diabetes patients utilizing a multigene strategy that comprised sequencing of every known gene linked to neonatal diabetes as well as 6q24 methylation tests. Their findings show that this testing technique successfully revealed a genetic reason in 80% of individuals, enabling a more precise pre- diction of the clinical course, anticipation of subsequent consequences, and the application of targeted medication [59,60].

We've come a long way in less than 200 years from not being able to measure blood glucose to being able to provide individuals with diabetes with continuous blood glucose monitoring coupled with continuous subcutaneous insulin infusion [61].

Most newborns with newly diagnosed diabetes require immediate insulin treatment to treat or avoid acute metabolic decompensation and to enable weight growth [62]. The cost-effectiveness of genetic testing for neonatal diabetes responsive to sulfonylurea (SU) has also been examined, in addition to the advantages of sulfonylurea therapy over insulin therapy that have already been covered [63].

The variance in individual and SU doses causes variations in the phenotypic profile and responsiveness to therapy for the same mutant genotype. Additionally, the prognosis of neurodevelopmental abnormalities in NDM with foci of encephalomalacia may be improved by SU only to a limited extent [64]. Our understanding of human illnesses has been considerably enhanced by developments in genomic medicine. Phenome, however, is not fully comprehended. The processes behind newborn disorders have been better understood thanks to high-resolution and multidimensional phenotypes, which may also help to improve treatment approaches [65].

The low prevalence of TNDM (1:200,000 to 1:400,000 live births) makes it difficult to gather information regarding its clinical characteristics, prognosis, and treatment. Until now, modest case studies have been used to characterize the clinical characteristics of 6q24 TNDM, some of which included patients without a molecularly verified diagnosis [14].

The majority of individuals with 6q24-TNDM had small-for-gestational-age (SGA) birth weights of 1930 g at 39 weeks of gestation or 2.5 SDS (standard deviation score),which most likely indicates an insulin deficit in utero [50].

The patents that have been filed for the use of natural substances to control diabetes lack comprehensive research [66].

4. Conclusions

Clinically, therapeutically, and genetically, neonatal diabetes continues to be a difficult condition to treat. There are no recommendations for managing diabetes during the first few months of birth; however, these two points are indisputable recommendations:

- The availability of genetic results (in a few weeks) significantly altered the short-and long-term management of these infants.
- These babies need insulin with adequate or high caloric intake to ensure satisfactory weight gain. Insulin administration can now partially mimic the pancreas physiology.

A molecular genetic diagnosis is advised for all NDM patients. These newborns with moderate or severe growth restrictions need early identification and medical therapy (constant insulin infusion and high caloric intake) to prevent catastrophic metabolic issues and allow for appropriate weight gain and brain development.

Regular follow-up is strongly advised, especially in the early years of childhood, since common illnesses may produce symptomatic hypoglycemia or recurrent hyperglycemia. CGM brought a benefit in this case because we were able to avoid the events of hypo- glycemia and act by decreasing the rate of insulin administration.

Author Contributions: A.L.B., L.M., A.L., C.E.F., I.I., A.A., A.C.P., M.N., V.V.L., C.I., C.M.M. and S.C.C. contributed equally with T.C. to this article. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with Declaration of Helsinki, and approved by the Ethics Committee of the Constanta Clinical Hospital, Romania (02/27 February 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data is available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Polak, M.; Cavé, H. Neonatal diabetes mellitus: A disease linked to multiple mechanisms. *Orphanet J. Rare Dis.* **2007**, *2*, 12. [CrossRef]
2. Fudvoye, J.; Farhat, K.; De Halleux, V.; Nicolescu, C.R. 6q24 Transient Neonatal Diabetes—How to Manage while Waiting for Genetic Results. *Front. Pediatr.* **2016**, *4*, 124. [CrossRef]
3. Demirbilek, H.; Arya, V.B.; Ozbek, M.N.; Houghton, J.A.L.; Baran, R.T.; Akar, M.; Tekes, S.; Tuzun, H.; Mackay, D.J.; Flanagan,S.E.; et al. Clinical characteristics and molecular genetic analysis of 22 patients with neonatal diabetes from the South-Easternregion of Turkey: Predominance of non-KATP channel mutations. *Eur. J. Endocrinol.* **2015**, *172*, 697–705. [CrossRef]
4. Temple, I.K.; Shield, J.P.H. Transient neonatal diabetes, a disorder of imprinting. *J. Med. Genet.* **2002**, *39*, 872–875. [CrossRef]
5. Grulich-Henn, J.; Wagner, V.; Thon, A.; Schober, E.; Marg, W.; Kapellen, T.M.; Haberland, H.; Raile, K.; Ellard, S.; Flanagan, S.E.; et al. Entities and frequency of neonatal diabetes: Data from the diabetes documentation and quality management system (DPV). *Diabet. Med.* **2010**, *27*, 709–712. [CrossRef]
6. Ashcroft, F.M.; Puljung, M.C.; Vedovato, N. Neonatal Diabetes and the K ATP Channel: From Mutation to Therapy. *Trends Endocrinol. Metab.* **2017**, *28*, 377–387. [CrossRef] [PubMed]
7. Babenko, A.P.; Polak, M.; Cavé, H.; Busiah, K.; Czernichow, P.; Scharfmann, R.; Bryan, J.; Aguilar-Bryan, L.; Vaxillaire, M.; Froguel, P. Activating Mutations in the *ABCC8* Gene in Neonatal Diabetes Mellitus. *N. Engl. J. Med.* **2006**, *355*, 456–466. [CrossRef][PubMed]
8. Al-Matary, A.; Hussain, M.; Nahari, A.; Ali, J. Permanent neonatal diabetes mellitus. *Am. J. Case Rep.* **2012**, *13*, 143–145. [CrossRef]
9. Hattersley, A.; Bruining, J.; Shield, J.; Njolstad, P.; Donaghue, K.C. The diagnosis and management of monogenic diabetes inchildren and adolescents. *Pediatr. Diabetes* **2009**, *10* (Suppl. S12), 33–42. [CrossRef] [PubMed]

10. Von Mühlendah, K.E.; Herkenhoff, H. Long-Term Course of Neonatal Diabetes. *N. Engl. J. Med.* **1995**, *333*, 704–708. [CrossRef]
11. Brink, S.J. Insulin Past, Present, and Future: 100 Years from Leonard Thompson. *Diabetology* **2022**, *3*, 117–158. [CrossRef]
12. Mustafa, M.; Ramdas, N.; Elhalik, M.; Faquih, A. Transient Neonatal Diabetes Mellitus with the Rare Association of Nonsuppura- tive Sialadenitis and Genetic Defects in 6q24. *Case Rep. Pediatr.* **2021**, *2021*, 5901898. [CrossRef] [PubMed]
13. Garin, I.; Edghill, E.L.; Akerman, I.; Rubio-Cabezas, O.; Rica, I.; Locke, J.M.; Maestro, M.A.; Alshaikh, A.; Bundak, R.; del Castillo, G.; et al. Recessive mutations in the *INS* gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 3105–3110. [CrossRef] [PubMed]
14. Docherty, L.E.; Kabwama, S.; Lehmann, A.; Hawke, E.; Harrison, L.; Flanagan, S.E.; Ellard, S.; Hattersley, A.T.; Shield, J.P.H.; Ennis,S.; et al. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype–phenotype correlation in an international cohort of patients. *Diabetologia* **2013**, *56*, 758–762. [CrossRef] [PubMed]
15. Beltrand, J.; Busiah, K.; Vaivre-Douret, L.; Fauret, A.L.; Berdugo, M.; Cavé, H.; Polak, M. Neonatal Diabetes Mellitus. *Front. Pediatr.* **2020**, *8*, 540178. [CrossRef]
16. Temple, I.K.; Mackay, D.J.G. Diabetes Mellitus, 6q24- Related Transient Neonatal. In *Gene Reviews*; Adam, M.P., Mirzaa, G.M., Pagon, R.A., Wallace, S.E., Bean, L.J.H., Gripp, G.W., Amemiya, A., Eds.; University of Washington: Seattle, WA, USA, 2005.
17. Kamiya, M.; Judson, H.; Okazaki, Y.; Kusakabe, M.; Muramatsu, M.; Takada, S.; Takagi, N.; Arima, T.; Wake, N.; Kamimura, K.;et al. The cell cycle control gene ZAC/PLAGL1 is imprinted--a strong candidate gene for transient neonatal diabetes. *Hum. Mol.Genet.* **2000**, *9*, 453–460. [CrossRef]
18. Barz, T.; Hoffmann, A.; Panhuysen, M.; Spengler, D. Peroxisome Proliferator-Activated Receptor γ Is a Zac Target Gene Mediating Zac Antiproliferation. *Cancer Res.* **2006**, *66*, 11975–11982. [CrossRef]
19. Arima, T.; Drewell, R.A.; Arney, K.L.; Inoue, J.; Makita, Y.; Hata, A.; Oshimura, M.; Wake, N.; Surani, M.A. A conserved imprintingcontrol region at the HYMAI/ZAC domain is implicated in transient neonatal diabetes mellitus. *Hum. Mol. Genet.* **2001**, *10*, 1475–1483. [CrossRef]
20. Shield, J.P. Neonatal Diabetes: New Insights into Aetiology and Implications. *Horm. Res. Paediatr.* **2000**, *53* (Suppl. S1), 7–11. [CrossRef]
21. Abacı, A.; Razi, C.H.; Özdemir, O.; Hızlı, S.; Kıslal, F.; Agras, P.I.; Kabakuş, N. Neonatal Diabetes Mellitus Accompanied by Diabetic Ketoacidosis and Mimicking Neonatal Sepsis: A Case Report. *J. Clin. Res. Pediatr. Endocrinol.* **2010**, *2*, 131–133. [CrossRef]
22. Metz, C.; Cavé, H.; Bertrand, A.M.; Deffert, C.; Gueguen-Giroux, B.; Czernichow, P.; Polak, M. Neonatal diabetes mellitus:Chromosomal analysis in transient and permanent cases. *J. Pediatr.* **2002**, *141*, 483–489. [CrossRef]
23. Flanagan, S.E.; Patch, A.-M.; Mackay, D.J.; Edghill, E.L.; Gloyn, A.L.; Robinson, D.; Shield, J.P.; Temple, K.; Ellard, S.; Hattersley, A.T. Mutations in ATP-Sensitive K+ Channel Genes Cause Transient Neonatal Diabetes and Permanent Diabetes in Childhood orAdulthood. *Diabetes* **2007**, *56*, 1930–1937. [CrossRef]
24. Suzuki, S.; Koga, M.; Amamiya, S.; Nakao, A.; Wada, K.; Okuhara, K.; Hayano, S.; Sarhat, A.R.; Takahashi, H.; Matsuo, K.; et al. Glycated albumin but not HbA1c reflects glycaemic control in patients with neonatal diabetes mellitus. *Diabetologia* **2011**, *54*, 2247–2253. [CrossRef]
25. Little, R.R.; Rohlfing, C.L.; Hanson, S.E.; Schmidt, R.L.; Lin, C.-N.; Madsen, R.W.; Roberts, W.L. The Effect of Increased Fetal Hemoglobin on 7 Common Hb A1c Assay Methods. *Clin. Chem.* **2012**, *58*, 945–947. [CrossRef]
26. Mihai, D.A.; Stefan, D.S.; Stegaru, D.; Bernea, G.E.; Vacaroiu, I.A.; Papacocea, T.; Lupus¸oru, M.O.D.; Nica, A.E.; Stiru, O.; Dragos, D.; et al. Continuous glucose monitoring devices: A brief presentation (Review). *Exp. Ther. Med.* **2021**, *23*, 174. [CrossRef] [PubMed]
27. Millman, G.C. Fanaroff and Martin's neonatal-perinatal medicine diseases of the fetus and infant, 8th edn, Vols I and II. *Arch. Dis. Child Fetal Neonatal Ed.* **2006**, *91*, F468.
28. Sarıcı, S.; Alpay, F.; Dündaröz, M.; Gökçay, E. Neonatal Diabetes Mellitus: Patient Report and Review of the literature. *J. Pediatr. Endocrinol. Metab.* **2001**, *14*, 451–454. [CrossRef]
29. Bharucha, T.; Brown, J.; McDonnell, C.; Gebert, R.; McDougall, P.; Cameron, F.; Werther, G.; Zacharin, M. Neonatal diabetes mellitus: Insulin pump as an alternative management strategy. *J. Paediatr. Child Health* **2005**, *41*, 522–526. [CrossRef] [PubMed]
30. Beardsall, K.; Vanhaesebrouck, S.; Ogilvy-Stuart, A.L.; Vanhole, C.; Vanweissenbruch, M.; Midgley, P.; Thio, M.; Cornette, L.; Ossuetta, I.; Palmer, C.R.; et al. Validation of the continuous glucose monitoring sensor in preterm infants. *Arch. Dis. Child.—Fetal Neonatal Ed.* **2013**, *98*, F136–F140. [CrossRef] [PubMed]
31. Beardsall, K.; Ogilvy-Stuart, A.L.; Ahluwalia, J.; Thompson, M.; Dunger, D.B. The continuous glucose monitoring sensor in neonatal intensive care. *Arch. Dis. Child.—Fetal Neonatal Ed.* **2005**, *90*, F307–F310. [CrossRef]
32. Harris, D.L.; Battin, M.R.; Weston, P.J.; Harding, J.E. Continuous Glucose Monitoring in Newborn Babies at Risk of Hypoglycemia. *J. Pediatr.* **2010**, *157*, 198–202.e1. [CrossRef]
33. Tiberi, E.; Cota, F.; Barone, G.; Perri, A.; Romano, V.; Iannotta, R.; Romagnoli, C.; Zecca, E. Continuous glucose monitoring in preterm infants: Evaluation by a modified Clarke error grid. *Ital. J. Pediatr.* **2016**, *42*, 29. [CrossRef] [PubMed]
34. Uettwiller, F.; Chemin, A.; Bonnemaison, E.; Favrais, G.; Saliba, E.; Labarthe, F. Real-Time Continuous Glucose Monitoring Reduces the Duration of Hypoglycemia Episodes: A Randomized Trial in Very Low Birth Weight Neonates. *PLoS ONE* **2015**, *10*,e0116255. [CrossRef] [PubMed]

35. Signal, M.; Le Compte, A.; Harris, D.; Weston, P.J.; Harding, J.E.; Chase, J.G. Impact of Retrospective Calibration Algorithms on Hypoglycemia Detection in Newborn Infants Using Continuous Glucose Monitoring. *Diabetes Technol. Ther.* **2012**, *14*, 883–890.[CrossRef]
36. McKinlay, C.J.D.; Chase, J.G.; Dickson, J.; Harris, D.L.; Alsweiler, J.M.; Harding, J.E. Continuous glucose monitoring in neonates: A review. *Matern. Health Neonatol. Perinatol.* **2017**, *3*, 18. [CrossRef]
37. Galderisi, A.; Facchinetti, A.; Steil, G.M.; Ortiz-Rubio, P.; Cavallin, F.; Tamborlane, W.V.; Baraldi, E.; Cobelli, C.; Trevisanuto, D. Continuous Glucose Monitoring in Very Preterm Infants: A Randomized Controlled Trial. *Pediatrics* **2017**, *140*, e20171162. [CrossRef]
38. Wackernagel, D.; Dube, M.; Blennow, M.; Tindberg, Y. Continuous subcutaneous glucose monitoring is accurate in term and near-term infants at risk of hypoglycaemia. *Acta Paediatr.* **2016**, *105*, 917–923. [CrossRef] [PubMed]
39. Harris, D.L.; Battin, M.R.; Williams, C.E.; Weston, P.J.; Harding, J.E. Cot-Side Electro-Encephalography and Interstitial Glucose Monitoring during Insulin-Induced Hypoglycaemia in Newborn Lambs. *Neonatology* **2009**, *95*, 271–278. [CrossRef] [PubMed]
40. Phillip, M.; Battelino, T.; Atlas, E.; Kordonouri, O.; Bratina, N.; Miller, S.; Biester, T.; Stefanija, M.A.; Muller, I.; Nimri, R.; et al. Nocturnal Glucose Control with an Artificial Pancreas at a Diabetes Camp. *N. Engl. J. Med.* **2013**, *368*, 824–833. [CrossRef]
41. Le Compte, A.; Chase, J.G.; Lynn, A.; Hann, C.; Shaw, G.; Wong, X.-W.; Lin, J. Blood Glucose Controller for Neonatal Intensive Care: Virtual Trials Development and First Clinical Trials. *J. Diabetes Sci. Technol.* **2009**, *3*, 1066–1081. [CrossRef]
42. Le Compte, A.J.; Lynn, A.M.; Lin, J.; Pretty, C.G.; Shaw, G.M.; Chase, J.G. Pilot study of a model-based approach to blood glucose control in very-low-birthweight neonates. *BMC Pediatr.* **2012**, *12*, 117. [CrossRef]
43. Beardsall, K.; Pesterfield, C.L.; Acerini, C.L. Neonatal diabetes and insulin pump therapy. *Arch. Dis. Child.—Fetal Neonatal Ed.* **2011**, *96*, F223–F224. [CrossRef]
44. Roth, M.; Lehmann, T.; Kloos, C.; Schmidt, S.; Kellner, C.; Wolf, G.; Müller, N. Metabolic Control, Diabetic Complications and Drug Therapy in a Cohort of Patients with Type 1 and Type 2 Diabetes in Secondary and Tertiary Care between 2004 and 2019. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2631. [CrossRef]
45. Urakami, T. The Advanced Diabetes Technologies for Reduction of the Frequency of Hypoglycemia and Minimizing the Occurrence of Severe Hypoglycemia in Children and Adolescents with Type 1 Diabetes. *J. Clin. Med.* **2023**, *12*, 781. [CrossRef]
46. Iwata, N.; Asui, R.; Mizumoto, H.; Hata, D. Management of a case of transient neonatal diabetes mellitus using continuous glucose monitoring. *Clin. Pediatr. Endocrinol.* **2020**, *29*, 77–80. [CrossRef] [PubMed]
47. Galderisi, A.; Trevisanuto, D.; Russo, C.; Hall, R.; Bruschettini, M. Continuous glucose monitoring for the prevention of morbidity and mortality in preterm infants. *Cochrane Database Syst. Rev.* **2021**, *2021*, CD013309. [CrossRef]
48. Soni, A.; Wright, N.; Agwu, J.; Timmis, A.; Drew, J.; Kershaw, M.; Moudiotis, C.; Regan, F.; Williams, E.; Wan, J.; et al. A practical approach to continuous glucose monitoring (rtCGM) and FreeStyle Libre systems (isCGM) in children and young people with Type 1 diabetes. *Diabetes Res. Clin. Pract.* **2022**, *184*, 109196. [CrossRef] [PubMed]
49. Kalogeropoulou, M.-S.; Iglesias-Platas, I.; Beardsall, K. Should continuous glucose monitoring be used to manage neonates at risk of hypoglycaemia? *Front. Pediatr.* **2023**, *11*, 1115228. [CrossRef]
50. Zugravu, C.; Nanu, M.I.; Moldovanu, F.; Arghir, O.C.; Mihai, C.M.; Oţelea, M.R.; Cambrea, S.C. The Influence of Perinatal Education on Breastfeeding Decision and Duration. *Int. J. Child Health Nutr.* **2018**, *7*, 74–81. [CrossRef]
51. Lemelman, M.B.; Letourneau, L.; Greeley, S.A.W. Neonatal Diabetes Mellitus: An Update on Diagnosis and Management. *Clin. Perinatol.* **2018**, *45*, 41–59. [CrossRef] [PubMed]
52. Uchida, N.; Ohnishi, T.; Kojima, T.; Takahashi, T.; Makita, Y.; Fukami, M.; Shibata, H.; Hasegawa, T.; Ishii, T. Relapsing 6q24-related transient neonatal diabetes mellitus with insulin resistance: A case report. *Clin. Pediatr. Endocrinol.* **2020**, *29*, 179–182. [CrossRef] [PubMed]
53. Yorifuji, T.; Higuchi, S.; Hosokawa, Y.; Kawakita, R. Chromosome 6q24-related diabetes mellitus. *Clin. Pediatr. Endocrinol.* **2018**, *27*, 59–65. [CrossRef] [PubMed]
54. Bonfanti, R.; Iafusco, D.; Rabbone, I.; Diedenhofen, G.; Bizzarri, C.; Patera, P.I.; Reinstadler, P.; Costantino, F.; Calcaterra, V.; Iughetti, L.; et al. Differences between transient neonatal diabetes mellitus subtypes can guide diagnosis and therapy. *Eur. J. Endocrinol.* **2021**, *184*, 575–585. [CrossRef] [PubMed]
55. Besser, R.E.; Flanagan, S.E.; Mackay, D.G.; Temple, I.; Shepherd, M.H.; Shields, B.M.; Ellard, S.; Hattersley, A.T. Prematurity and Genetic Testing for Neonatal Diabetes. *Pediatrics* **2016**, *138*, e20153926. [CrossRef] [PubMed]
56. Babonji, M.; Kalantan, K.; Alenezi, F.; Alruwaili, R.; Bantan, E.; Al Saleh, R.; Abdulrahim, N.; Abuyabis, R.; Lsarefi, Z.; Aljubairah, W.; et al. Epidemiology and Clinical Phenotypes and Presentations of Neonatal Diabetes. *J. Health Sci.* **2021**, *1*, 133–139. [CrossRef]
57. Yorifuji, T.; Matsubara, K.; Sakakibara, A.; Hashimoto, Y.; Kawakita, R.; Hosokawa, Y.; Fujimaru, R.; Murakami, A.; Tamagawa, N.; Hatake, K.; et al. Abnormalities in chromosome 6q24 as a cause of early-onset, non-obese, non-autoimmune diabetes mellitus without history of neonatal diabetes. *Diabet. Med.* **2015**, *32*, 963–967. [CrossRef]
58. March, C.A.; Libman, I.M.; Becker, D.J.; Levitsky, L.L. From Antiquity to Modern Times: A History of Diabetes Mellitus and Its Treatments. *Horm. Res. Paediatr.* **2022**, *95*, 593–607. [CrossRef]
59. Kocova, M. Genetic spectrum of neonatal diabetes. *Balk. J. Med. Genet.* **2021**, *23*, 5–15. [CrossRef]
60. Gore, R.H.; Nikita, M.E.; Newton, P.G.; Carter, R.G.; Reyes-Bautista, J.; Greene, C.L. Duplication 6q24: More Than Just Diabetes. *J. Endocr. Soc.* **2020**, *4*, bvaa027. [CrossRef]

61. De Franco, E.; Flanagan, S.E.; AL Houghton, J.; Allen, H.L.; Mackay, D.J.; Temple, I.K.; Ellard, S.; Hattersley, A.T. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: An international cohort study. *Lancet* **2015**, *386*,957–963. [CrossRef]
62. Beardsall, K.; Thomson, L.; Guy, C.; Iglesias-Platas, I.; van Weissenbruch, M.M.; Bond, S.; Allison, A.; Kim, S.; Petrou, S.; Pantaleo,B.; et al. Real-time continuous glucose monitoring in preterm infants (REACT): An international, open-label, randomised controlled trial. *Lancet Child Adolesc. Health* **2021**, *5*, 265–273. [CrossRef] [PubMed]
63. Sood, S.; Landreth, H.; Bustinza, J.; Chalmers, L.; Thukaram, R. Neonatal Diabetes: Case Report of a 9-Week-Old Presenting Dia-betic Ketoacidosis Due to an Activating ABCC8 Gene Mutation. *J. Investig. Med. High Impact Case Rep.* **2017**, *5*, 2324709617698718. [CrossRef] [PubMed]
64. Wu, B.; Xu, W. Case report: Neonatal diabetes mellitus caused by KCNJ11 mutation presenting with intracranial hemorrhage.
Front. Neurol. **2023**, *14*, 1072078. [CrossRef] [PubMed]
65. Xiao, T.; Dong, X.; Lu, Y.; Zhou, W. High-Resolution and Multidimensional Phenotypes Can Complement Genomics Data to Diagnose Diseases in the Neonatal Population. *Phenomics* **2022**, *3*, 204–215. [CrossRef]
66. DasNandy, A.; Virge, R.; Hegde, H.V.; Chattopadhyay, D. A review of patent literature on the regulation of glucose metabolism bysix phytocompounds in the management of diabetes mellitus and its complications. *J. Integr. Med.* **2023**, *21*, 226–235. [CrossRef]

Diagnostics

MDPI

Article

Helicobacter pylori Infection in Children: A Possible Reason for Headache?

Ancuta Lupu [1], Cristina Gavrilovici [1], Vasile Valeriu Lupu [1,*], Anca Lavinia Cianga [1,*], Andrei Tudor Cernomaz [2], Iuliana Magdalena Starcea [1,*], Cristina Maria Mihai [3,*], Elena Tarca [4], Adriana Mocanu [1] and Silvia Fotea [5]

[1] Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania
[2] III-rd Medical Department, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania
[3] Pediatrics, Faculty of General Medicine, Ovidius University, 900470 Constanta, Romania
[4] Department of Surgery II—Pediatric Surgery, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania
[5] Medical Department, Faculty of Medicine and Pharmacy, "Dunarea de Jos" University of Galati, 800008 Galati, Romania
* Correspondence: valeriulupu@yahoo.com (V.V.L.); ancalaviniacianga@gmail.com (A.L.C.); magdabirm@yahoo.com (I.M.S.); cristina2603@yahoo.com (C.M.M.)

Abstract: (1) Background: The correlation between infection with *Helicobacter pylori* (*H. pylori*) and headache has been argued and explored for a long time, but a clear association between the simultaneous presence of the two in children has not been established yet. In this study, we aimed to explore this relationship in children from the Northeast region of Romania. (2) Methods: A retrospective study exploring the correlation between children having *H. pylori* infection and headache or migraine was conducted on a batch of 1757 children, hospitalized over 3 years in a pediatric gastroenterology department in Northeast Romania. (3) Results: A total of 130 children of both sexes had headache. From 130 children, 54 children (41.5%) also presented *H. pylori* infection. A significant association between headache and *H. pylori* infection ($\chi 2$; $p < 0.01$) was noticed. (4) Conclusions: More studies are needed on this relationship, and we emphasize the importance of further analyses, as they present great clinical importance for both prompt diagnosis and treatment.

Keywords: headache; *Helicobacter pylori*; children; migraine

Citation: Lupu, A.; Gavrilovici, C.; Lupu, V.V.; Cianga, A.L.; Cernomaz, A.T.; Starcea, I.M.; Mihai, C.M.; Tarca, E.; Mocanu, A.; Fotea, S. *Helicobacter pylori* Infection in Children: A Possible Reason for Headache? *Diagnostics* **2023**, *13*, 1293. https://doi.org/10.3390/diagnostics13071293

Academic Editor: Cristina Oana Marginean

Received: 1 March 2023
Revised: 25 March 2023
Accepted: 27 March 2023
Published: 29 March 2023

1. Introduction

Headache represents a common complaint among the pediatric population, which, as well as in adults, is frequently underdiagnosed, although it affects the quality of life. The prevalence of migraine, one of the most common types of primary headache, is estimated at approximately 9% among the pediatric population, and a perpetual increase in its incidence has been registered over the last three decades. Among the indicative factors of an increased incidence of headache, Anttila et al. mention sleep deprivation, an increase in the use of information technology, and soft drink consumption [1]. In children, migraine and tension-type headache usually occur simultaneously with a mixed symptomatology [2]. They are usually considered to be self-limited conditions, but they can persist from childhood to adulthood, affecting the quality of life [3]. Being a burdensome condition, headache plays an important role in mental and physical health, and in children, it can impair school performance or lead to social isolation [4,5]. Thus, headache can interfere negatively with the entire education path in childhood and adolescence [6]. In children, headache can present through neurobehavioral symptoms such as agitation, sleep disturbances, irritability, and trouble concentrating [7]. Various factors such as sleep disorders, genetics, environmental factors such as humidity, light, or noise, severe trauma, and menstruation have been indicated as risk factors and possible triggers of migraine

headaches [8]. The literature suggests that there may be a significant connection between *Helicobacter pylori* (*H. pylori*) infection and headaches. However, though this association has been explored in adults, there is still a significant gap in data regarding this issue in the pediatric population [9].

H. pylori is a gram-negative, microaerophilic, spiral bacterium with increased motility as a result of the presence of multiple unipolar flagella [10]. It generates urease and colonizes the mucus layer adjacent to the gastric mucosa, usually being responsible for gastrointestinal impairments such as chronic active gastroenteritis, infection, gastric andduodenal ulcer, and, more rarely, stomach cancer [11]. The bacterium possesses adaptive characteristics that allows the body's survival in an acidic environment. It produces urease that consequently converts urea into bicarbonate and ammonium and leads to theneutralization of the gastric acid [12]. However, numerous studies claim that infection with *H. pylori* may be the result of various extra-digestive conditions such as neurological, cardiovascular, metabolic, hematologic, ocular, or dermatological ones.

Along with various extra-digestive impairments, the relationship between the infec-tion with *H. pylori* and neurological manifestations such as mild cognitive impairment, migraine, or Alzheimer's disease have been extensively studied, but there are no clear results concerning the pathophysiology of the process [13]. However, it was shown that the systemic effects of the infection with *H. pylori* are the result of the modulation of the gut–brain axis (GBA), which consists of a two-way signaling pathway between the gastro-intestinal tract (GIT) and the brain, and which plays a pivotal role in infections andadditional clinical outcomes [14–16].

Among the stated hypotheses, *H. pylori* infection may trigger a host immune response to the presence of bacteria and a consequent release of vasoactive substances [17]. The pathological process encompasses immunological events such as migration of lymphocytic, monocytic, and neutrophilic invasion into the gastric mucosa and submucosa, along withthe release of chemokines or pro-inflammatory cytokines such as IL-6, IL-1β, TNF-α, or IL-8 at the site of infection [18,19]. Moreover, *H. pylori* infection disturbs the balance in the microbiota, influences the host–pathogen interaction, and plays an important role in themodulation of the gastric microenvironment, thus causing changes in homeostasis [20,21].

H. pylori type I cagA-positive strains are also thought to have the ability to induce a significant release of proinflammatory substances by the gastric mucosa, leading consequently to systemic vasospasms [22]. As described in the literature, for patients with *H. pylori* infection who complain of headache, bacteria eradication might improve the symptoms or reduce the migraine-related disability level [19].

There exist multiple pharmaceutical treatment plans for managing both digestive and extra-digestive infections and diseases caused by *H. pylori*. Timely and precise identification of *H. pylori* plays a critical role in the effective treatment and eradication of the bacterium. Due to *H. pylori*'s specific localization in the gastric mucus layer, pharmacotherapy must be effective in penetrating this layer to prevent *H. pylori* colonization. As such, the medication used must be able to penetrate the gastric mucosal layer [23].

The data on children in Romania regarding *H. pylori* infection reveals a declining trend that might be the result of improving socio-economic conditions [24]. However, Yuan et al. highlighted in their review that despite advances in medical science, *H. pylori* infection continues to exhibit a high incidence rate among children worldwide, emphasizing the significance of this infection [25].

Although the data regarding the link between *H. pylori* infection and headache have been explored for many years, and the results of this association are controversial, we present the results we obtained on a pediatric population in Northeast Romania.

2. Materials and Methods

We performed a retrospective study on 1757 children, hospitalized over 3 years in a pediatric gastroenterology department in "St. Maria" Emergency Hospital for Children in Iasi, Romania, complaining of symptoms suggestive of gastric or duodenal ulcer. Thus,

according to Jones et al.'s recommendations from 2017 [26], all the 1757 patients underwent superior digestive endoscopy. With a treatment that was likely to be offered, biopsies and cultures were taken for the examined patients. The diagnostic of infection with *H. pylori* was established by having *H. pylori*-positive gastritis on histopathology examination, along with positive cultures. For these patients, we focused on the association between the *Helicobacter pylori* infection and the presence of headache/migraine. Out of the 1757 patients of both sexes, we selected based on the anamnestic findings those who complained of migraine or headache at admission. To assess the importance of headache, the Migraine Disability Assessment Test (MIDAS) along with the Visual Analogue Scale (VAS) were utilized.

We excluded children who previously received eradication treatment of *H. pylori*, those who previously had treatment with acetaminophen or antibiotics, children with evidence of bleeding of the gastro-intestinal tract at endoscopy, those who complained of headache or migraine during previous hospitalizations or had a medical past history of headache/migraine, patients with gastrointestinal disorders known to be associated with headache such as inflammatory bowel syndrome, celiac disease, or functional abdominal pain, and patients with a history of drug use, including H2 blockers, antibiotics, or protonpump inhibitors, within 4 weeks [27–29].

Based on the available information in the literature, migraine was defined as the presence of severe and recurrent headache attacks, along with neurological and autonomic symptoms. The diagnostic criteria for pediatric migraine were realized according to the second edition of the International Classification of Headache Disorders (ICHD-2) [30].

All patients enrolled underwent upper gastrointestinal endoscopic examination with intravenous sedation, and video pediatric gastroduodenoscopes from Pentax and Olympus were used. For children under 10 years old, the procedure was performed under general anesthesia. Biopsies were collected from the antrum and gastric corpus during endoscopy for rapid urease testing and histological and bacteriological examination [27–29].

Informed consent was taken from all caregivers, and the study was approved by the "St. Mary" Emergency Hospital for Children Ethics Committee's (no.31490/29.10.2021).

Data were extracted from the hospital database, patient observation charts, endoscopy results, and discharge papers. IBM SPSS 17.0 platform, GraphPad Prism, and Microsoft Excel were used to analyze the data.

3. Results

From the 1757 patients, 542 had infection with *H. pylori*. We reported the structure of the study group in Table 1.

Table 1. Study group presenting or not presenting *H. pylori* infection.

Infection with *H. pylori*		**%**
absent	1215	69.2
present	542	30.8
Headache		
absent	1627	92.6
present	130	7.4
Sex		
female	1210	68.9
male	547	31.1
Area of living		
urban	643	36.6
rural	1114	63.4

The main symptoms that led to admission to the pediatric gastroenterology clinic and that later resulted in the diagnosis of gastritis were represented in order of frequency by: abdominal pain in 1664 cases (94.7%), nausea in 668 cases (38.0%), vomiting in 468 cases (27.2%), inappetence in 243 cases (13.8%), heartburn in 144 cases (8.2%), headache in 130 cases (7.4%), vertigo in 87 cases (5.0%), constipation in 57 cases (3.2%), abdominal flatulence in 56 cases (6.2%), asthenia in 26 cases (1.5%), and early satiety in 17 cases (1.0%).

Among the non-specific symptoms, we found a strongly significant association be- tween headache and infection with *H. pylori* ($\chi 2$; $p < 0.01$) (Table 2). Of the 130 childrenwho had headaches, 54 children (41.5%) were also diagnosed with infection with *H. pylori*.

Table 2. Estimated parameters in testing the association between *H. pylori* infection and headache.

	Headache (+)	**Headache (−)**
HP (+)	54	488
HP (−)	76	1139
	p value = 0.006	

HP—*Helicobacter pylori*.

Important differences between sex-stratified subgroups regarding *H. pylori* infection and headache were also noticed. Our evaluation showed that the prevalence of headache was almost five times higher in girls, out of the total number of 130 cases (83.1% females vs. 16.9% males). (Table 3).

Table 3. Differences between sex-stratified subgroups.

	HP (+)	**HP (−)**	**Headache (+)**	**Headache (−)**
Sex				
male	145	402	22	525
female	397	813	108	1102
		p = 0.0002 chi-squared		

HP—*Helicobacter pylori*.

Moreover, regarding the area of living, we noticed a higher number of children from rural areas—104 children (80%), compared to 26 children (20%) from urban areas—among all cases with headache.

Subsequently, we evaluated the environmental distribution of *H. pylori* (+) and ( ) patients. The distribution of children with *H. pylori* (+) according to the area of living revealed a frequency of 75.3% in rural environments compared to 24.7% in urban areas (Figure 1).

In addition, the exploration of the age distribution of all the patients from the initial batch showed a mean value of 13.19 years (SD = +3.501) (Figure 2).

Considering the population analyzed, which is represented by hospital-referred pedi- atric patients with clinical pictures suggestive of gastritis, there seems to be a significant difference in the odds of having chronic headache complaints between the *H. pylori* positive and negative subgroups: odds ratio = 1.658 (95% confidence interval [CI]: 1.15–2.38)—as indicated by a post-hoc binary logistic regression model including the presence of headache as the dependent variable and *H. pylori* infection status, gender, and living conditions as covariates. The computed power was 77% (given the sample size of 1757 and an effect size of 0.065), and the goodness of fit of the model was deemed low. More data would be useful, but given the retrospective nature of our analysis, no viable solution to enlarge the data pool was identified.

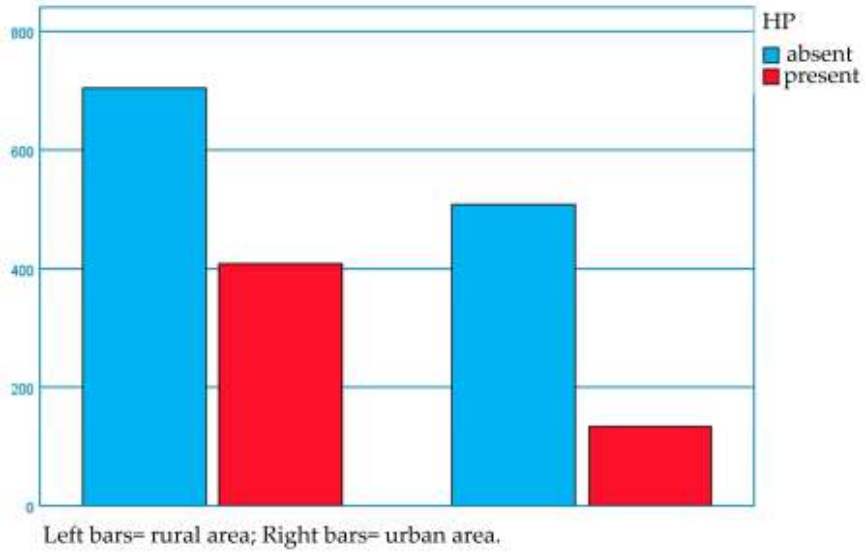


Figure 1. Environmental distribution of patients with or without infection with *H. pylori*.

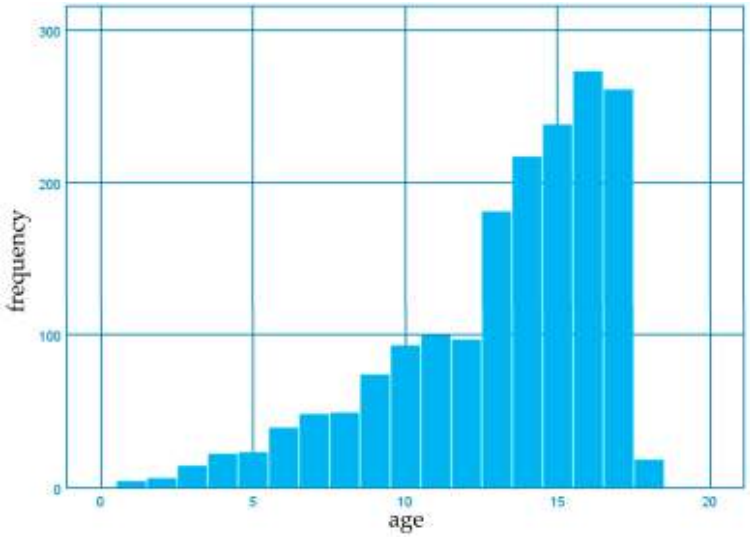


Figure 2. Age distribution of patients with or without infection with *H. pylori*.

4. Discussion

Migraine is a common condition consisting of primary headaches with a prevalence of 15% in Western societies [31]. Secondary headache is frequently reported by patients with various gastrointestinal disorders such as gastroesophageal reflux disease, inflammatory bowel syndrome, constipation, functional abdominal pain, or *H. pylori* infection, but the potential causal link remains unclear [32,33]. However, in recent years, research has focused on the implication of *H. pylori* activity in the pathogenesis of migraine, as this microorganism was identified as a cause for multiple extra digestive manifestations [22]. It has been hypothesized that the recurrence of headaches following *H. pylori* infection may be due to the systemic vasospastic effects of proinflammatory substances that are released by the infected gastric mucosa [34]. Other authors indicate the production of platelet-activating factor in *H. pylori* infections and claim that the migraine attacks may be the result of the high level of serotonin released from platelets [35]. Eradication of *H. pylori* infection resulted in a significant reduction in the intensity, frequency, and duration of migraine attacks [36,37]. However, the studies conducted on the correlation between *H. pylori* infection and headache have provided mixed and controversial results.

Kikui et al. showed in their study that in comparison to non-migraine individuals ofsimilar characteristics, migraine sufferers exhibit a higher prevalence of gastrointestinal

comorbidities—specifically, increased odds of irritable bowel syndrome (adjusted oddsratio (95% CI: 3.8 (2.7 to 5.4)), heartburn (3.6 (95% CI 2.8 to 4.7)), gastroesophageal reflux disease (3.5 (2.5, 4.8)), ulcers (3.1 (95% CI 2.0 to 4.8)), frequent diarrhea (3.1 (95% CI 2.3 to 4.1)), and chronic constipation (2.5 (95% CI 1.9 to 3.3)) [38].

Our study found a strongly significant association between headache and infection with *H. pylori* ($\chi 2$; $p < 0.01$). Out of the 130 children who complained of headaches, 54 of them (41.5%) also had concomitant *H. pylori* infection. Thereby, a high prevalence of infection with *H. pylori* in patients with headache/migraine is indicated. These results are in agreement with the findings of Cavestro et al., who conducted an impressive cross-sectional study on the relationship between *H. pylori* infection and headache and found a significant association between these two entities ($p = 0.009$) [39]. Moreover, in their case-control study, Yiannopoulou et al. presumed the same association and found that *H. pylori* infection prevalence was significantly higher in 49 patients with headache than in 51 control subjects ($p = 0.016$) [40]. In their study on 70 patients, Hosseinzadeh et al. also showed that the prevalence of migraine was significantly correlated with the IgG and IgM titer against *H. pylori* (p 0.048 and p 0.03, respectively) [41]. ≤ ≤

In addition, the eradication treatment for *H. pylori* infection proved a beneficial effect on the patients suffering from migraine compared to controls in a study conducted by Tunca et al. [36]. Comparable results were obtained in 2012 by Faraji et al., who showedthat their patients with migraine who received *H. pylori* eradication treatment presented a lower headache-related disability level than those in the placebo batch. The mechanismsinvolved could be linked to oxidative stress and nitric oxide imbalance secondary to acute inflammation caused by *H. pylori* infection [42]. The same improvement was obtained byKarkelis et al., who evaluated a number of 65 children suffering from headache and mi- graine and discovered that 17 of them had concomitant *H. pylori* infection. After completing the anti-Helicobacter infection therapy, a complete resolution of migraine symptoms wasnoticed for all the patients in the study [43].

All these studies agree with the results reported by Gasbarrini et al. in 1997 and 1998, who described both an association between *H. pylori* infection and headache, as well as a significant alleviation of the intensity of headache along with the eradication of *H. pylori* infection [44,45]. In their case-control study, Hassan et al. obtained a similar result with a significant prevalence of infection with *H. pylori* in 77 migraine patients ($p < 0.001$, OR = 3.439), but at the same time, they obtained no correlation between *H. pylori* infection and migraine attacks, migraine disability assessment test, or the visual analogue scale. In addition, *H. pylori* infection did not represent a trigger for the migraine attacks or a risk factor for an increased frequency of headache episodes [46].

Su et al. also described in their meta-analysis of five case-control studies that *H. pylori* infection was positive in approximately 45% of patients with migraine compared to a prevalence rate of 33% among healthy controls (OR = 1.92, 95%CI: 1.05–3.51, $p = 0.001$). Moreover, the infection rate of *H. pylori* was higher in Asian patients with migraine, but the same could not be established for European ones (OR = 3.48 and 1.19, respectively) [47].

In contradiction with the results above, Lee et al. found a higher frequency of *H. pylori* infection in patients with migraines or headaches than in the control groups, but no statistical significance was obtained ($p = 0.51$) [48]. In Iran, a study was conducted on84 patients that revealed a significant correlation between the severity of headache and the IgG antibody. However, there was no statistically significant difference observed in levels of IgG in migraine versus control subjects. On the other hand, they observed a statistical significance in the IgM antibody titer against *H. pylori* among the patients with migraine compared to those in the control batches ($p = 0.004$) [49]. The role of interleukin-10 was also speculated, on one hand because its elevation was associated with both migraine and *H. pylori* infection (cagA-positive strains, in particular), and on the other hand, due to the fact that sumatriptan (5-HT1D receptor agonist) decreases the levels of this cytokine during a migraine attack [50,51].

In another study conducted on 31 children complaining of migraine and abdominal pain, an impressive high prevalence of esophagitis (41.9%), antral gastritis (38.7%), duo- denitis (87.1%), and corpus gastritis (51.6%) was found. However, only seven patients out of the total had simultaneous *H. pylori* infection, and no association between migraine and *H. pylori* infection could be made [52]. In the same manner, Ciancarelli et al. evaluated 30 subjects suffering from migraine and found that for only 16.7% of them, the infection with *H. pylori* was confirmed, leading to the absence of a certain association between infection with *H. pylori* and migraine [53].

Interestingly, a retrospective study from Turkey performed on 526 subjects with mi- graine described that the infection with *H. pylori*, as a chronic infection, can be more aggressive and may represent one of the risk factors of the apparition and development of matter lesions in these patients. Here, Ocal et al. found that white matter lesions (WMLs) were present in 178 (33.8%) *H. pylori*-positive subjects ($p < 0.05$), and more than that, there was a 2.5-fold higher incidence of WMLs on the brain MRIs of migraine patients with *H. pylori* infection [54]. In an impressive cross-sectional study from 2021 that covered 489,753 participants, Welander et al. found a significant association between migraine and *H. pylori* infection when entered separately, but with other gastro-intestinal condi- tions added to the same adjusted model, the statistical significance could not be validated (OR = 1.34, $p = 0.024$) [55].

Having a multifactorial susceptibility with hormonal, genetic, and environmental factors each playing different, but important roles, headache affects over 17% of females and only 5–8% of males [56]. In our study, we also found that out of the 130 patients who complained of headache, 108 were females (83.1%), and only 22 were males (16.9%). Hormati et al. also described in their research on 341 patients the presence of a higher prevalence of migraine among females ($p = 0.003$) [57]. Akbari et al. described in their research performed on 305 patients with dyspepsia that the prevalence of migraine was significantly higher in female patients compared to male patients (48.9% vs. 35.5%, respec- tively, $p < 0.010$) [58]. This idea may be explained by the fact that girls are, firstly, usually more aware of their symptoms; secondly, they are prone to encounter more headache episodes along with the debut of menstrual cycles; and thirdly, females may present a more important bacterial load.

Regarding the area of living, the results of our study describe an increased incidence of headache/migraine among the pediatric population living in the rural area (80%) than those from the urban one (20%). These results are similar to the environmental distribution among the *H. pylori*-positive patients in our study, where 75.3% came from rural environments, whereas 24.7% lived in urban areas. These facts are consistent with Martin et al., who found that along with females and white people, the individuals residing in rural areas were more likely to suffer from headache than their respective comparison batches [59]. This hypothesis may be explained by the lack of specialist care in rural regions, thus leading to a lower adherence to headache or migraine management and treatment.

Knowing that *H. pylori* may lead to a low luminal pH by decreasing the bicarbonate secretion and increasing the acid secretion, this microorganism is recognized for its capacity to weaken the mucosa in the areas of gastric metaplasia and to make the mucosa more vulnerable to acid secretion. With many findings that associated the presence of *H. pylori* infection with migraine, Hormati et al. even postulated that a low value of the gastric pH can represent a trigger for headache, whereas the treatment with proton pump inhibitor (PPI) drugs may contribute to a significant improvement in both the severity and frequency of migraine attacks [57]. Another hypothesis raised by Lileikyte et al. involves the role of *H. pylori* in the development of vestibular migraine through irritation of the respiratory mucosa by the gastric acid, with subsequent inflammation or direct local infection caused by the presence of *H. pylori*, but these interesting affirmations need further explorations [60]. Further, individuals who suffer from migraines may have an increased risk of de- veloping vitamin B12 deficiency due to the use of non-steroidal anti-inflammatory drugs for acute symptom relief and an increased incidence of active *H. pylori* infection [61]. In their article, Urits et al. describe that many individuals with migraines also experience gastro-intestinal damage, and *H. pylori* infection

further impairs vitamin B12 absorption by destroying gastric parietal cells and reducing the availability of intrinsic factors [62].

Recently developed antimigraine drugs, such as anti-calcitonin gene-related peptideantibodies (CGRP) and monoclonal antibodies, offer a promising breakthrough in thetreatment of migraine. The antimigraine mechanism of action of these drugs is similar tothat of a kynurenic acid analogue, which can eliminate nitroglycerin-induced hyperalge- sia by increasing CGRP expression. The kynurenine pathway, which is involved in the metabolism of L-tryptophan, is known to be altered in functional gastrointestinal diseases that are associated with migraines. In consequence, targeting this pathway may be an effective approach for treating both migraine and functional gastrointestinal diseases [63].

The present study showed a significant association between the infection with *H. pylori* and headache, but this relationship needs further studies. Although findings about the correlations between *H. pylori* and headache pathogenesis have been accumulating, the existing data do not completely amount to an unequivocal conclusion. However, there are certain effects of *H. pylori* infection, such as a decrease in food sensitivity, a lack of changes in plasma levels of thiobarbituric acid-reactive substances, and nitric oxide metabolites in infected patients compared to control subjects, and there is a similar prevalence of infection with *H. pylori* in patients with migraines compared to healthy subjects. These effects could be interpreted as valid arguments against *H. pylori* being considered a riskfactor for migraines [36,47,53].

The need for establishing a definite association between headache and the *H. pylori* infection remains of great importance. Furthermore, our study has its limitations, such as a lack of paraclinical investigations consequent to the irregularity of the funding availability, the impossibility of performing real-time PCR for testing the antimicrobial susceptibility in *H. pylori* infection, along with the inability to conduct a long-term follow-up of the children due to their refractoriness.

5. Conclusions

Currently, there is sufficient evidence that correlates the increased frequency of mi- graine or headache with various gastro-intestinal disorders, compared to the general pedi- atric population. However, no clear association between *H. pylori* infection and headache was established to present. The gut–brain axis needs further exploration, as it is indicated in playing an important role between the *H. pylori* infection—migraine relationship.

It is important to note that further investigations should be carried out to evaluate the effectiveness of *H. pylori* eradication on the severity of headaches, the long-term clinical implications of this potential relationship, the assessment of multiple strains of *H. pylori* in children with headache, and the ethnicity of the participants under study. Furthermore, the variation in *H. pylori* in different regions should also be considered as a significant factor in future evaluations. We are certain that a better understanding of this association between headache and gastrointestinal disorders in children is of great clinical importance for both prompt diagnosis and treatment.

Author Contributions: C.G., A.T.C., E.T., I.M.S., C.M.M. and A.M. contributed equally with A.L. to this article. Conceptualization, A.L., A.L.C. and V.V.L.; methodology, C.M.M. and C.G.; software, A.T.C. and I.M.S.; formal analysis, A.T.C., E.T. and A.M.; investigation, A.L., A.L.C. and V.V.L.; data curation, A.T.C.; writing—original draft preparation, A.L., A.T.C., A.L.C. and E.T.; writing—review and editing, C.G., I.M.S., C.M.M., V.V.L., A.M. and S.F.; visualization, A.M. and I.M.S.; supervision, C.M.M. and C.G.; project administration, S.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the “St. Maria” Emergency Hospital for Children, Iasi, Romania (31490/29.10.2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank the Endoscopy Department and the colleagues for their involvementin managing these patients.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Anttila, P.; Metsähonkala, L.; Sillanpää, M. Long-term Trends in the Incidence of Headache in Finnish Schoolchildren. *Pediatrics* **2006**, *117*, e1197–e1201. [CrossRef]
2. Blankenburg, M.; Schroth, M.; Braun, S. Chronic headache in children and adolescents. *Klin. Padiatr.* **2019**, *231*, 14–20.
3. Teleanu, R.I.; Vladacenco, O.; Teleanu, D.M.; Epure, D.A. Treatment of Pediatric Migraine: A Review. *Maedica* **2016**, *11*, 136–143.
4. Bigal, M.E.; Lipton, R.B. The epidemiology, burden, and comorbidities of migraine. *Neurol. Clin.* **2009**, *27*, 321–334. [CrossRef]
5. Wöber-Bingöl, Ç. Epidemiology of Migraine and Headache in Children and Adolescents. *Curr. Pain Headache Rep.* **2013**, *17*, 341. [CrossRef]
6. Nieswand, V.; Richter, M.; Gossrau, G. Epidemiology of Headache in Children and Adolescents—Another Type of Pandemia. *Curr. Pain Headache Rep.* **2020**, *24*, 62. [CrossRef]
7. Gazerani, P. Migraine and Mood in Children. *Behav. Sci.* **2021**, *11*, 52. [CrossRef]
8. Deleu, D.; Hanssens, Y.; Worthing, E.A. Symptomatic and prophylactic treatment of migraine: A critical reappraisal. *Clin. Neuropharmacol.* **1998**, *21*, 267–279.
9. Le Gal, J.; Michel, J.F.; Rinaldi, V.E.; Spiri, D.; Moretti, R.; Bettati, D.; Romanello, S.; Berlese, P.; Lualdi, R.; Boizeau, P.; et al. Association between functional gastrointestinal disorders and migraine in children and adolescents: A case-control study. *Lancet Gastroenterol. Hepatol.* **2016**, *1*, 114–121. [CrossRef]
10. Wang, Y.-H.; Lv, Z.-F.; Zhong, Y.; Liu, D.-S.; Chen, S.-P.; Xie, Y. The internalization of Helicobacter pylori plays a role in the failureof H. *pylori eradication. Helicobacter* **2016**, *22*, e12324. [CrossRef]
11. Pinessi, L.; Savi, L.; Pellicano, R.; Rainero, I.; Valfre, W.; Gentile, S.; Cossotto, D.; Rizzetto, M.; Ponzetto, A. Chronic Helicobacter Pylori Infection and Migraine: A Case-Control Study. *Headache* **2000**, *40*, 836–839. [CrossRef]
12. Figueiredo, C.; Machado, J.C.; Yamaoka, Y. Pathogenesis of Helicobacter pylori Infection. *Helicobacter* **2005**, *10*, 14–20. [CrossRef]
13. Pellicano, R.; Ianiro, G.; Fagoonee, S.; Settanni, C.R.; Gasbarrini, A. Review: Extragastric diseases and Helicobacter pylori. *Helicobacter* **2020**, *25*, e12741. [CrossRef]
14. Kountouras, J.; Zavos, C.; Polyzos, S.A.; Deretzi, G. The gut-brain axis: Interactions between Helicobacter pylori and enteric and central nervous systems. *Ann. Gastroenterol.* **2015**, *28*, 506.
15. Budzyński, J. Brain-gut axis in the pathogenesis of Helicobacter pylori infection. *World J. Gastroenterol.* **2014**, *20*, 5212–5225. [CrossRef]
16. Baj, J.; Forma, A.; Flieger, W.; Morawska, I.; Michalski, A.; Buszewicz, G.; Sitarz, E.; Portincasa, P.; Garruti, G.; Flieger, M.; et al. Helicobacter pylori Infection and Extragastric Diseases—A Focus on the Central Nervous System. *Cells* **2021**, *10*, 2191. [CrossRef]
17. Savi, L.; Ribaldone, D.; Fagoonee, S.; Pellicano, R. Is Helicobacter pylori the infectious trigger for headache?: A review. *Infect. Disord.—Drug Targets* **2014**, *13*, 313–317. [CrossRef]
18. Tsai, H.-F.; Hsu, P.-N. Interplay between Helicobacter pylori and immune cells in immune pathogenesis of gastric inflammationand mucosal pathology. *Cell. Mol. Immunol.* **2010**, *7*, 255–259. [CrossRef]
19. Arzani, M.; Jahromi, S.R.; Ghorbani, Z.; Vahabizad, F.; Martelletti, P.; Ghaemi, A.; Sacco, S.; Togha, M.; On behalf of the School of Advanced Studies of the European Headache Federation (EHF-SAS). Gut-brain Axis and migraine headache: A comprehensivereview. *J. Headache Pain* **2020**, *21*, 15. [CrossRef]
20. Mayer, E.A.; Tillisch, K.; Bradesi, S. Review article: Modulation of the brain–gut axis as a therapeutic approach in gastrointestinal disease. *Aliment. Pharmacol. Ther.* **2006**, *24*, 919–933. [CrossRef]
21. Sticlaru, L.; Stăniceanu, F.; Cioplea, M.; Nichita, L.; Bastian, A.; Micu, G.; Popp, C. Dangerous Liaison: Helicobacter pylori, Ganglionitis, and Myenteric Gastric Neurons: A Histopathological Study. *Anal. Cell. Pathol.* **2019**, *2019*, 3085181. [CrossRef] [PubMed]
22. Gasbarrini, A.; Gabrielli, M.; Fiore, G.; Candelli, M.; Bartolozzi, F.; De Luca, A.; Cremonini, F.; Franceschi, F.; Di Campli, C.; Armuzzi, A.; et al. Association Between Helicobacter Pylori Cytotoxic Type I Caga-Positive Strains and Migraine with Aura. *Cephalalgia* **2000**, *20*, 561–565. [CrossRef] [PubMed]
23. Ranjbar, R.; Behzadi, P.; Farshad, S. Advances in diagnosis and treatment of Helicobacter pylori infection. *Acta Microbiol. et Immunol. Hung.* **2017**, *64*, 273–292. [CrossRef]
24. Corojan, A.L.; Dumitraș cu, D.; Ciobanca, P.; Leucuta, D. Prevalence of Helicobacter pylori infection among dyspeptic patients in Northwestern Romania: A decreasing epidemiological trend in the last 30 years. *Exp. Ther. Med.* **2020**, *20*, 3488–3492. [CrossRef]

25. Yuan, C.; Adeloye, D.; Luk, T.T.; Huang, L.; He, Y.; Xu, Y.; Ye, X.; Yi, Q.; Song, P.; Rudan, I.; et al. The global prevalence of and factors associated with Helicobacter pylori infection in children: A systematic review and meta-analysis. *Lancet Child Adolesc. Health* **2022**, *6*, 185–194. [CrossRef]
26. Jones, N.L.; Koletzko, S.; Goodman, K.; Bontems, P.; Cadranel, S.; Casswall, T.; Czinn, S.; Gold, B.D.; Guarner, J.; Elitsur, Y.; et al. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). *J. Pediatr. Gastroenterol. Nutr.* **2017**, *64*, 991–1003. [CrossRef] [PubMed]
27. Lupu, A.; Miron, I.C.; Cianga, A.L.; Cernomaz, A.T.; Lupu, V.V.; Munteanu, D.; Ghica, D.C.; Fotea, S. The Relationship between Anemia and Helicobacter Pylori Infection in Children. *Children* **2022**, *9*, 1324. [CrossRef]
28. Lupu, A.; Miron, I.C.; Cianga, A.L.; Cernomaz, A.T.; Lupu, V.V.; Gavrilovici, C.; Stârcea, I.M.; Tarca, E.; Ghica, D.C.; Fotea, S. The Prevalence of Liver Cytolysis in Children with Helicobacter pylori Infection. *Children* **2022**, *9*, 1498. [CrossRef] [PubMed]
29. Lupu, A.; Miron, I.C.; Cernomaz, A.T.; Gavrilovici, C.; Lupu, V.V.; Starcea, I.M.; Cianga, A.L.; Stana, B.; Tarca, E.; Fotea, S. Epidemiological Characteristics of Helicobacter pylori Infection in Children in Northeast Romania. *Diagnostics* **2023**, *13*, 408. [CrossRef] [PubMed]
30. Olesen, J. The international classification of headache disorders, 2nd edn (ICDH-II). *J. Neurol. Neurosurg. Psychiatry* **2004**, *75*, 808–811. [CrossRef] [PubMed]
31. Stewart, W.F.; Lipton, R.B.; Celentano, D.D.; Reed, M.L. Prevalence of migraine headache in the United States. Relation to age,income, race, and other sociodemographic factors. *JAMA* **1992**, *267*, 64–69. [CrossRef] [PubMed]
32. Holtmann, G.; Goebell, H.; Holtmann, M.; Talley, N.J. Dyspepsia in healthy blood donors: Pattern of symptoms and association with Helicobacter pylori. *Dig. Dis. Sci.* **1994**, *39*, 1090–1098. [CrossRef] [PubMed]
33. Imanieh, M.H.; Dehghani, S.M.; Haghighat, M.; Irani, M.; Yousefi, M. Migraine headache and acid peptic diseases in children. *Iran. Red Crescent Med. J.* **2009**, *11*, 181–183.
34. Pacifico, L.; Anania, C.; Osborn, J.F.; Ferraro, F.; Chiesa, C. Consequences of Helicobacter pylori infection in children. *World J. Gastroenterol.* **2010**, *16*, 5181–5194. [CrossRef]
35. Panconesi, A.; Sicuteri, R. Headache induced by serotonergic agonists—A key to the interpretation of migraine pathogenesis? *Cephalalgia* **1997**, *17*, 3–14. [CrossRef]
36. Tunca, A.; Turkay, C.; Tekin, O.; Kargili, A.; Erbayrak, M. Is Helicobacter pylori infection a risk factor for migraine? A case-control study. *Acta Neurol. Belg.* **2004**, *104*, 161–164.
37. Bradbeer, L.; Thakkar, S.; Liu, A.; Nanan, R. Childhood headache and *H. pylori*: A possible association. *Aust. Fam. Physician* **2013**, *42*, 134–136. [PubMed]
38. Kikui, S.; Chen, Y.; Ikeda, K.; Hasebe, M.; Asao, K.; Takeshima, T. Comorbidities in patients with migraine in Japan: A cross-sectional study using data from National Health and Wellness Survey. *BMJ Open* **2022**, *12*, e065787. [CrossRef]
39. Cavestro, C.; Prandi, G.; Manildo, M.; Martini, S.; Genovesi, C.; Premoli, A.; Fraire, F.; Neri, L.; Mandrino, S.; Ferrero, M.; et al. A cross-sectional study on the association between Helicobacter pylori infection and headache. *Neurol. Sci.* **2022**, *43*, 6031–6038. [CrossRef]
40. Yiannopoulou, K.G.; Efthymiou, A.; Karydakis, K.; Arhimandritis, A.; Bovaretos, N.; Tzivras, M. Helicobacter pylori infection as an environmental risk factor for migraine without aura. *J. Headache Pain* **2007**, *8*, 329–333. [CrossRef]
41. Hosseinzadeh, M.; Khosravi, A.; Saki, K.; Ranjbar, R. Evaluation of Helicobacter pylori infection in patients with commonmigraine headache. *Arch. Med. Sci.* **2011**, *5*, 844–849. [CrossRef] [PubMed]
42. Faraji, F.; Zarinfar, N.; Zanjani, A.T.; Morteza, A. The effect of Helicobacter pylori eradication on migraine: A randomized, doubleblind, controlled trial. *Pain Physician* **2012**, *15*, 495–498. [PubMed]
43. Karkelis, S.; Papadaki-Papandreou, O.; Lykogeorgou, M.; Papandreou, T.; Lianou, L.; Panayotou, I.; Roma, E.; Chrousos, G. 667 Helicobacter Pylori Infection and Headache in Children and Adolescents. *Pediatr. Res.* **2010**, *68*, 340. [CrossRef]
44. Gasbarrini, A.; De Luca, A.; Fiore, G.; Gambrielli, M.; Franceschi, F.; Ojetti, V.; Torre, E.S.; Gasbarrini, G.; Pola, P.; Giacovazzo, M. Beneficial effects of Helicobacter pylori eradication on migraine. *Hepato-Gastroenterology* **1998**, *45*, 765–770. [PubMed]
45. Gasbarrini, A.; De Luca, A.; Fiore, G.; Franceschi, F.; Ojetti, V.; Torre, E.S.; Di Campli, C.; Candelli, M.; Pola, M.S.R.; Tondi, P.; et al. Primary Headache and Helicobacter Pylori. *Int. J. Angiol.* **1998**, *7*, 310–312. [CrossRef] [PubMed]
46. Hassan, A.; Mehany, D.; Eldin, H.G.; Abdelghaffar, M.; Abdelbaky, H.A.; Kamal, Y.S.; Hussein, M. Helicobacter pylori infection in migraine headache: A true association or an innocent bystander? *Int. J. Neurosci.* **2022**, 1–6. [CrossRef] [PubMed]
47. Su, J.; Zhou, X.Y.; Zhang, G.X. Association between helicobacter pylori infection and migraine: A meta-analysis. *World J. Gastroenterol.* **2014**, *20*, 14965–14972. [CrossRef] [PubMed]
48. Lee, S.H.; Lee, J.J.; Kwon, Y.; Kim, J.H.; Sohn, J.H. Clinical Implications of Associations between Headache and Gastrointestinal Disorders: A Study Using the Hallym Smart Clinical Data Warehouse. *Front. Neurol.* **2017**, *8*, 526. [CrossRef]
49. Ansari, B.; Basiri, K.; Meamar, R.; Chitsaz, A.; Nematollahi, S. Association of Helicobacter pylori antibodies and severity ofmigraine attack. *Iran. J. Neurol.* **2015**, *14*, 125–129.
50. Kang, J.W.; Shin, Y.I. The role of interleukin 10 in the associations between migraine and Helicobacter pylori infection. *PainPhysician* **2013**, *16*, E450. [CrossRef]
51. Munno, I.; Marinaro, M.; Bassi, A.; Cassiano, M.; Causarano, V.; Centonze, V. Immunological aspects in migraine: Increase ofIL-10 plasma levels during attack. *Headache* **2001**, *41*, 764–767. [CrossRef]

52. Mavromichalis, I.; Zaramboukas, T.; Giala, M.M. Migraine of gastrointestinal origin. *Eur. J. Pediatr.* **1995**, *154*, 406–410. [CrossRef][PubMed]
53. Ciancarelli, I.; Di Massimo, C.; Ciancarelli, M.G.T.; De Matteis, G.; Marini, C.; Carolei, A. Helicobacter Pylori Infection and Migraine. *Cephalalgia* **2002**, *22*, 222–225. [CrossRef] [PubMed]
54. Öcal, S.; Öcal, R.; Suna, N. Relationship between Helicobacter pylori infection and white matter lesions in patients with migraine.
BMC Neurol. **2022**, *22*, 187. [CrossRef]
55. Welander, N.Z.; Olivo, G.; Pisanu, C.; Rukh, G.; Schiöth, H.B.; Mwinyi, J. Migraine and gastrointestinal disorders in middle andold age: A UK Biobank study. *Brain Behav.* **2021**, *11*, e2291. [CrossRef]
56. Cámara-Lemarroy, C.R.; Rodriguez-Gutierrez, R.; Monreal-Robles, R.; Marfil-Rivera, A. Gastrointestinal disorders associated with migraine: A comprehensive review. *World J. Gastroenterol.* **2016**, *22*, 8149–8160. [CrossRef] [PubMed]
57. Hormati, A.; Akbari, N.; Sharifipour, E.; Hejazi, S.A.; Jafari, F.; Alemi, F.; Mohammadbeigi, A. Migraine and gastric disorders: Arethey associated? *J. Res. Med. Sci.* **2019**, *24*, 60. [CrossRef] [PubMed]
58. Akbari, N.; Hormati, A.; Sharifipour, E.; Hejazi, S.A.; Jafari, F.; Mousavi-Aghdas, S.A.; Golzari, S.E. Migraine, dyspepsia, and Helicobacter pylori: Zeroing in on the culprit. *Iran. J. Neurol.* **2019**, *18*, 19–24. [CrossRef]
59. Martin, B.C.; Dorfman, J.H.; McMillan, J.A.; McMillan, C.A. Prevalence of migraine headache and association with sex, age, race,and rural/urban residence: A population-based study of Georgia Medicaid recipients. *Clin. Ther.* **1994**, *16*, 855–872.
60. Lileikytė, V.; Brasas, K.; Vaitkus, A.; Žvirblienė, A. Is vestibular migraine really a separate form of migraine? *Med. Hypotheses* **2022**, *165*, 110880. [CrossRef]
61. Martami, F.; Ghorbani, Z.; Abolhasani, M.; Togha, M.; Meysamie, A.; Sharifi, A.; Jahromi, S.R. Comorbidity of gastrointestinaldisorders, migraine, and tension-type headache: A cross-sectional study in Iran. *Neurol. Sci.* **2017**, *39*, 63–70. [CrossRef] [PubMed]
62. Urits, I.; Yilmaz, M.; Bahrun, E.; Merley, C.; Scoon, L.; Lassiter, G.; An, D.; Orhurhu, V.; Kaye, A.D.; Viswanath, O. Utilization ofB12 for the treatment of chronic migraine. *Best Pract. Res. Clin. Anaesthesiol.* **2020**, *34*, 479–491. [CrossRef] [PubMed]
63. Fila, M.; Chojnacki, J.; Pawlowska, E.; Szczepanska, J.; Chojnacki, C.; Blasiak, J. Kynurenine Pathway of Tryptophan Metabolismin Migraine and Functional Gastrointestinal Disorders. *Int. J. Mol. Sci.* **2021**, *22*, 10134. [CrossRef] [PubMed]

CORRELATION BETWEEN VITAMIN D DEFICIENCY AND TYPE 1 DIABETES IN CHILDREN

Tatiana Chisnoiu[1,2], Cristina Maria Mihai[1,2,*], Adriana Luminita Bălașa[1,2], Alexandru Cosmin Pantazi[1,*], Antonio Andrusca[1,2], Bianca Maria Constantin[2], Andreea Dalila Nedelcu[3], Simona Claudia Cambrea[1]

[1] Faculty of Medicine, "Ovidius" University, 900470 Constanta, Romania
[2] Pediatrics, County Clinical Emergency Hospital of Constanta, 900591 Constanta, Romania
[3] Balneal and Rehabilitation Sanatorium of Techirghiol, Romania

[*] Corresponding authors: Cristina Maria Mihai (cristina2603@yahoo.com);
Alexandru Cosmin Pantazi (pantazi.cosmin@365.univ-ovidius.ro)

ABSTRACT

Background: Vitamin D is an organic compound with an important role in bone metabolism and ability to modulate the immune function. Epidemiology research have recently suggested vitamin D's role in the etiology of type 1 and type 2 diabetes in children. Vitamin D controls insulin secretion in pancreatic islets and insulin sensitivity in several peripheral metabolic organs by acting through the vitamin D receptor (VDR). Recent studies have reported an increased prevalence of vitamin D deficiency among patients diagnosed with type 1 diabetes. **The aim of the study** is to identify the correlation between vitamin D deficiency and the frequency, severity, and control of type 1 diabetes in children. **Material and Methods:** The retrospective study was performed on 40 subjects with type 1 diabetes, which associated vitamin D deficiency at the onset of the disease or during the investigation period, at Pediatric Clinic of Constanta County Clinical Emergency Hospital. The strategy used involved the collection of certain parameters from the observation sheets, in order to evaluate, compare and illustrate the correlation between vitamin D deficiency and type 1 diabetes. **Results:** Male subjects represented the majority of cases (55%). The seasonal incidence may be explained by exposure to a reduced amount of ultraviolet radiation in the cold season, which contributes to the decrease in the level of 25-hydroxyvitamin D. Viral infections had an increased incidence in the winter, which may contribute to the onset of type 1 diabetes. An optimal level of serum 25-hydroxyvitamin D was found in 17% cases, insufficient level in 63% cases and deficiency in 20% cases. In evolution, 24 cases had poor glycemic control, registering values higher than 7% of glycosylated hemoglobin. The dosage of 25 hydroxyvitamin D in these subjects indicated vitamin D deficiency in 7 cases and insufficiency in 17 cases. None of the patients with a glycosylated hemoglobin value >7 had an optimal 25-hydroxyvitamin D level. Out of 16 patients with optimal glycosylated hemoglobin values (<7%), 2 cases presented vitamin D deficiency, 6 cases insufficient level, and 8 cases had an optimal value of 25-hydroxyvitamin D. **Conclusions:** Vitamin D deficiency is associated with the evolution of type 1 diabetes in children. The evaluation of vitamin D levels is a potential disease-modifying factor in type I diabetes therapeutic management.

Keywords: type 1 diabetes, vitamin D, deficiency, children

INTRODUCTION

Type 1 diabetes is a complex metabolic condition

characterized by the loss of insulin production capacity. This deficiency is due to the destruction of pancreatic β-cells. Insulin- dependent diabetes is frequently diagnosed in childhood and young adulthood, the age at presentation having a bimodal distribution (a first peak at 4-6 years and a second peak at 10- 14 years) [1]. More than one million children and adolescents are diagnosed with type 1 diabetes mellitus. Worldwide approximately 132,600 children and adolescents may develop type 1 diabetes annually [2].

Vitamin D, called calciferol, is part of a group of vitamins (D1, D2, D3, D4, D5, D6, D7) with an important role in the metabolism of calcium and phosphorus, which are found in nature in the form of provitamins. [3]

Hypovitaminosis D and vitamin D deficiency, regardless of narrow definitions, have a higher prevalence worldwide at any age. In pediatrics, US data from the National Health and Nutrition Examination Survey cohort indicate a prevalence of vitamin D deficiency and hypovitaminosis D ranging from 9–18% to 51–61%, respectively [4,5].

The main action of vitamin D in the body is to regulate the concentration and ratio of calcium/phosphorus [6]. From absorption and transport to the use of these elements in the bones, vitamin D constantly intervenes through its hydroxylated compounds. Thus, any metabolic process involving calcium and phosphorus depends directly or indirectly on presence, quality, and functionality of vitamin D [7]. Recent studies on vitamin D have demonstrated significant interactions between vitamin D and immune system cells, both innate and adaptive [8].

The influence on the development of the immune system is determined by factors such as mode of delivery, host genotype, breastfeeding, and gut microbiome [9,10]. Children born by C-section have an increased risk for altered immune development [10]. Specific mechanisms are implied in the determinism of gut microbiome in diseases related to altered immune development and potential therapeutic targets for immune related-disorders [11,12]. Gut microbiota has potent modulators of immune cells function and a role in initiating autoimmune conditions [13-16]. Neonatal diabetes mellitus is encountered in infants under six months of age and newborns, with difficulties related to therapeutic management [17].

A relationship between type 1 diabetes and vitamin D deficiency has been reported [18,19]. There is evidence that vitamin D is important in preventing islet cell death and may be useful in improving the survival of islet cell grafts and improving insulin production. According to epidemiologic data, having enough vitamin D may help delay or prevent the onset of diabetes [20].

MATERIAL AND METHODS

Vitamin D deficiency has been associated with numerous autoimmune and inflammatory disorders, which is why it represents a problem of great interest in the field of health. The objective of this study is to identify a possible association between the level of 25 hydroxyvitamin D and type I diabetes in pediatric patients.

Vitamin D may influence the activity of the immune system, which induces the destruction of insulin-secreting pancreatic β- cells, in genetically susceptible children and may influence metabolic control during the disease. The present study aims to identify the correlation between vitamin D deficiency and the frequency, severity, and control of type 1 diabetes.

The retrospective study was performed on 40 patients with type 1 diabetes and vitamin D deficiency at the onset of the disease or during the investigation period, from Pediatric Clinic of Constanta County Clinical Emergency Hospital. The strategy used involved the collection of certain parameters from the observation sheets, in order to evaluate, compare and establish a possible association between type 1 diabetes and vitamin D deficiency in children.

RESULTS AND DISCUSSIONS

In 2012 and 2013, 2 cases of type 1 diabetes were registered, equivalent to 5%. In 2016, 2017, and 2021, 4 cases were confirmed
annually, equivalent to 10%. In 2018, 8 cases were registered (20% cases), and in 2019, a record was set, identifying 16 subjects (40% cases) (*Table 1*). The number of subjects with type 1 diabetes associated with low vitamin D varied annually.

Table 1. Distribution of cases by year

Year	Number of subjects
2012	2
2013	2
2016	4
2017	4
2018	8
2019	16
2021	4

The distribution of cases according to the season showed that in the winter 17 cases were registered (43%), in the spring the diagnosis was established in 13 cases (32%), whereas during the summer and autumn, the incidence decreased significantly, 7 cases (17%) respectively 3 cases (8%). The seasonal incidence may be explained by exposure to a reduced amount of ultraviolet radiation in the cold season, which contributes to the decrease in the level of 25-hydroxyvitamin D. Also, viral infections had an increased incidence in the winter season, which may contribute to the onset of type 1 diabetes. Considering the gender, the male subjects represented the majority (55% cases), while the females represented 45% cases (*Figure 1*).

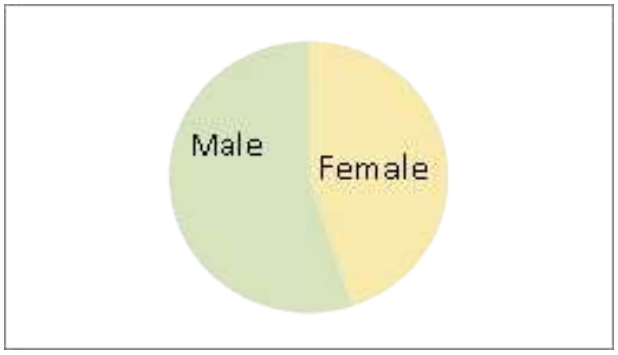


Figure 1. Distribution of cases by gender

According to the onset of the disease, ketoacidosis was identified in 15 cases (9 females and 6 males) equivalent to 37% cases; classic symptomatology was identified in 24 cases (9 females and 15 males), the equivalent of 60% cases, and incidental finding was identified in 1 male (3% cases).

In the studied group, the presence of a relevant family history was observed in 79% cases, a fact that supports the involvement of genetic factors in the occurrence of type 1 diabetes. Heredo-collateral antecedents identified were represented by diabetes, arthritis, thyroid, and cardiac pathology.

Alhonen et al. 2011 showed that diabetes and other autoimmune pathologies are frequently encountered in the extended families of diagnosed patients. The study included 300 families with at least one child diagnosed with insulin-dependent diabetes and 381 control families. According to the analysis, the risk of occurrence was significantly associated with a positive family history [21].

The autoimmune process that induces type I diabetes can also affect other organs.

Regarding rickets prophylaxis, 45% cases (18 subjects) performed rickets prophylaxis according to the recommended scheme, while 55% cases did not perform prophylaxis (14 subjects) or interrupted recommended therapy (8 subjects). Hypponen et al. 2001 suggested that performing rickets prophylaxis in the first year of life can be crucial in preventing diabetes in children [23].

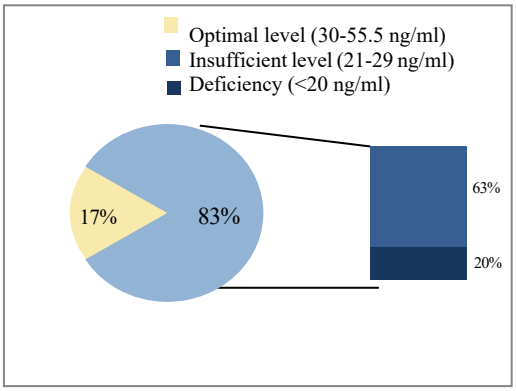


Figure 2. Distribution of cases by vitamin D levels

An optimal level of serum 25-hydroxyvitamin D (30-55.5 ng/ml) was observed in 17% cases; 63% cases (25 subjects) presented an insufficient level (21-29 ng/ml), and 20% cases (8 subjects) had values suggestive for vitamin deficiency (<20 ng/ml) (*Figure 2*).

The study performed by Svoren et al. in 2009 found that 15% of subjects with type I diabetes were deficient in 25-hydroxyvitamin D, and 61% presented insufficient levels [24].

The dynamic evaluation of the level of 25-hydroxyvitamin D led to the following analytical data: 20% (8 cases) presented an optimal level, 57% (23 cases) showed an insufficient level, and 23% (9 cases) presented values for deficiency. It was observed that 25-hydroxyvitamin D deficiency identified at the beginning of the diagnosis can also be maintained dynamically in certain cases.

In evolution, 24 cases had poor glycemic control, registering values higher than 7% of glycosylated hemoglobin. The dosage of 25 hydroxyvitamin D in these subjects indicated deficiency in 7 cases and insufficiency levels in 17 cases. None of the patients with a glycosylated hemoglobin value >7 had an optimal 25-hydroxyvitamin D level. Out of 16 patients with optimal glycosylated hemoglobin values (< 7%), 2 cases presented vitamin D deficiency, 6 cases insufficient level, and 8 cases had an optimal value of 25- hydroxyvitamin D.

Following a statistical analysis, no subject with HbA1c>7 recorded a 25- hydroxyvitamin D value between the reference parameters, while 49% cases with adequate glycemic control had an optimal level. Values of 25-hydroxyvitamin D between 21-29 ng/ml were recorded in 70% cases with HbA1c >7% and in 38% cases with HbA1c < 7%. Vitamin D deficiency was reported in 30% cases with poor control of type 1 diabetes, compared to 13% in subjects with optimal glycosylated hemoglobin.

In children at high genetic risk, vitamin D insufficiency may increase the risk of type 1 diabetes in the first years of life. Additionally, subjects with type 1 diabetes are frequently deficient in vitamin D. The proof of vitamin D supplementation and the maintenance of beta- cell activity in type 1 diabetes currently is not clear. Future large-scale studies are necessary to fully evaluate vitamin D's potential as a disease-modifying option in type 1 diabetes [25].

CONCLUSIONS

In the families of diagnosed patients, it was observed a significant history of autoimmune pathologies represented by autoimmune thyroiditis and celiac disease. The dosage of specific antibodies revealed the presence of anti-GAD antibodies in 70% cases, anti-IA-2 antibodies in 65% cases, and anti- IAA antibodies in 37% cases. These results support the autoimmune etiology of the disease and the involvement of genetic factors in the pathogenic mechanism.

Confirmation of the diagnosis was carried out mainly in the winter and spring season. During this period, due to exposure to a reduced amount of ultraviolet radiation, children frequently presented serum values of 25-hydroxyvitamin D below the optimal level.

At the time of diagnosis of type I diabetes, 83% cases associated a serum value of 25 hydroxyvitamin D below the recommended level. During the evolution of the disease, 79% cases recorded reduced serum values of 25-hydroxyvitamin D.

In evolution, by measuring glycosylated hemoglobin and 25-hydroxyvitamin D it was observed that inadequate glycemic control was associated with vitamin insufficiency in 70% cases and the presence of deficiency in 30% cases. Subjects with adequate glycemic control presented vitamin insufficiency in 38% cases, deficiency in 13% cases, and optimal levels of 25-hydroxyvitamin D in 49% cases.

Acknowledgements: CMM, ALB, ACP, VVL, AA, BMC, AL, ADN, SCC contributed equally with TC to this article.

REFERENCES

1. Latha Ganti, Javier Rosario, David Lebowitz, Javier Rosario, Ariel Vera. Step-Up to USMLE Step 2 CK 5th Edition, Wolters Kluwer Health (LWW), 2019
2. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, Aschner P, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018 Oct;19 Suppl 27(Suppl 27):7-19. doi: 10.1111/pedi.12773. PMID: 30226024; PMCID: PMC7521365.
3. Mohammadi S, Hajhashemy Z, Saneei P. Serum vitamin D levels in relation to type-2 diabetes and prediabetes in adults: a systematic review and dose-response meta-analysis of epidemiologic studies. Crit Rev Food Sci Nutr. 2022;62(29):8178-8198. doi: 10.1080/10408398.2021.1926220. Epub 2021 Jun 2. PMID: 34076544.
4. Mansbach JM, Ginde AA, Camargo CA Jr. Serum 25-hydroxyvitamin D levels among US children aged 1 to 11 years: do children need more vitamin D? Pediatrics. 2009 Nov;124(5):1404-10. doi: 10.1542/peds.2008-2041. Erratum in: Pediatrics. 2009 Dec;124(6):1709. PMID: 19951983; PMCID: PMC3765249.
5. Cashman KD, Dowling KG, Škrabáková Z, Gonzalez-Gross M, Valtueña J, et al. Vitamin D deficiency in Europe: pandemic? Am J Clin Nutr. 2016 Apr;103(4):1033-44. doi: 10.3945/ajcn.115.120873. Epub 2016 Feb 10. PMID: 26864360; PMCID: PMC5527850.
6. Elena Ionica, Marieta Costache. Biochimie generală, Vitamine și elemente minerale-volumul III, Editura Ars Docendi, 2004
7. Elena Cristea-Popa, Aurora Popescu, E. Truția, Veronica Dinu. Tratat de biochimie medicală-volumul I, Editura Medicală, 1991
8. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013 Jul 5;5(7):2502-21. doi: 10.3390/nu5072502. PMID: 23857223; PMCID: PMC3738984.
9. Zugravu C, Nanu MI, Moldovanu F, et al. The Influence of Perinatal Education on Breastfeeding Decision and Duration. Int J Child Health Nutr. 2018;7(2):74-81. doi:10.6000/1929-4247.2018.07.02.4
10. Lupu VV, Miron IC, Raileanu AA, et al. Difficulties in Adaptation of the Mother and Newborn via Cesarean Section versus Natural Birth—A Narrative Review. Life 2023, Vol 13, Page 300. 2023;13(2):300. doi:10.3390/LIFE13020300
11. Pantazi AC, Mihai CM, Balasa AL, Chisnoiu T, Lupu A, Frecus CE, Mihai L, Ungureanu A, Kassim MAK, Andrusca A, Nicolae M, Cuzic V, Lupu VV, Cambrea SC. Relationship between Gut Microbiota and Allergies in Children: A Literature Review. Nutrients. 2023; 15(11):2529, https://doi.org/10.3390/nu15112529
12. Lupu VV, Raileanu AA, Mihai CM et al. The Implication of the Gut Microbiome in Heart Failure. Cells 2023, Vol 12, Page 1158. 2023;12(8):1158, doi:10.3390/CELLS12081158
13. Lupu VV, Ghiciuc CM, Stefanescu G, et al. Emerging role of the gut microbiome in post-infectious irritable bowel syndrome: A literature review. World J Gastroenterol. 2023;29(21):3241-3256, doi:10.3748/WJG.V29.I21.3241
14. Lupu VV, Trandafir LM, Raileanu AA, Mihai CM, Morariu ID, Starcea IM, Mocanu A, Butnariu LI, Stoleriu G, Salaru DL, Chisnoiu T, Munteanu D, Mitrofan C, Lupu A. Advances in Understanding the Human Gut Microbiota and Its Implication in Pediatric Celiac Disease—A Narrative Review. Nutrients. 2023; 15(11):2499, doi:0.3390/nu15112499
15. Lupu VV, Jechel E, Mihai CM, Mitrofan EC, Lupu A, Starcea IM, Fotea S, Mocanu A, Ghica DC, Mitrofan C, Munteanu D, Salaru DL, Morariu ID, Ioniuc I. Connection between Celiac Disease and Systemic Lupus Erythematosus in Children—A Development Model of Autoimmune Diseases Starting from What We Inherit to What We Eat. Nutrients. 2023; 15(11):2535, doi:10.3390/nu15112535

16. Lupu A, Miron IC, Gavrilovici C, Raileanu AA, Starcea IM, Ioniuc I, Azoicai A, Mocanu A, Butnariu LI, Dragan F, Lupu VV. Pediatric Systemic Lupus Erythematous in COVID-19 Era. Viruses. 2023; 15(2):272. doi:10.3390/v15020272
17. Chisnoiu T, Balasa AL, Mihai L, Lupu A, Frecus CE, Ion I, Andrusca A, Pantazi AC, Nicolae M, Lupu VV, et al. Continuous Glucose Monitoring in Transient Neonatal Diabetes Mellitus—2 Case Reports and Literature Review. Diagnostics. 2023; 13(13):2271. https://doi.org/10.3390/diagnostics13132271
18. Snijder M, van Dam R, Visser M, Deeg D, Seidell J, Lips P. To: Mathieu C, Gysemans C, Giulietti A, Bouillon R (2005) Vitamin D and diabetes. Diabetologia 48:1247-1257. Diabetologia. 2006 Jan;49(1):217-8. doi: 10.1007/s00125-005-0047-9. Epub 2005 Dec 13. PMID: 16344926.
19. Greer RM, Rogers MA, Bowling FG, Buntain HM, Harris M, Leong GM, Cotterill AM. Australian children and adolescents with type 1 diabetes have low vitamin D levels. Med J Aust. 2007 Jul 2;187(1):59-60. doi: 10.5694/j.1326-5377.2007.tb01130.x. PMID: 17605716.
20. Penckofer S, Kouba J, Wallis DE, Emanuele MA. Vitamin D and diabetes: let the sunshine in. Diabetes Educ. 2008 Nov-Dec;34(6):939-40, 942, 944 passim. doi: 10.1177/0145721708326764. PMID: 19075078; PMCID: PMC2910714.
21. Alhonen S, Korhonen S, Tapanainen P, Knip M, Veijola R. Extended family history of diabetes and autoimmune diseases in children with and without type 1 diabetes. Diabetes Care. 2011 Jan;34(1):115-7. doi: 10.2337/dc10-1091. Epub 2010 Sep 27. PMID: 20876204; PMCID: PMC3005485.
22. Krzewska A, Ben-Skowronek I. Effect of Associated Autoimmune Diseases on Type 1 Diabetes Mellitus Incidence and Metabolic Control in Children and Adolescents. Biomed Res Int. 2016;2016:6219730. doi: 10.1155/2016/6219730. Epub 2016 Jul 20. PMID: 27525273; PMCID: PMC4971288.
23. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001 Nov 3;358(9292):1500-3. doi: 10.1016/S0140-6736(01)06580-1. PMID: 11705562.
24. Svoren BM, Volkening LK, Wood JR, Laffel LM. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr. 2009 Jan;154(1):132-4. doi: 10.1016/j.jpeds.2008.07.015. PMID: 19187735; PMCID: PMC2635941.
25. Infante M, Ricordi C, Sanchez J, Clare-Salzler MJ, Padilla N, Fuenmayor V, Chavez C, Alvarez A, Baidal D, Alejandro R, Caprio M, Fabbri A. Influence of Vitamin D on Islet Autoimmunity and Beta-Cell Function in Type 1 Diabetes. Nutrients. 2019 Sep 11;11(9):2185. doi: 10.3390/nu11092185. PMID: 31514368; PMCID: PMC6769474.

Journal of Environmental Protection and Ecology 20, No 3, 1496–1504 (2019)

Public health – environmental medicine

EVOLUTION OF SALMONELLOSIS IN CONSTANTA AREA IN CORRELATION WITH ENVIRONMENTAL FACTORS

S. HALICHIDIS[a], A. L. BALASA[b], E. V. IONESCU[a], M. G. ILIESCU[a], S. C. CAMBREA[a,c]*, L. C. PETCU[d]*, C. M. MIHAI[a]

[a]*Faculty of Medicine, 'Ovidius' University, 1st Aleea Universitatii, Campus Corp B, Constanta, Romania*
[b]*Constanta County Clinical Emergency Hospital 'Sf. Ap. Andrei', 145 Tomis Avenue, 900 591 Constanta, Romania*
[c]*Clinical Infectious Diseases Hospital of Constanta, 100 Ferdinand Avenue, 900 178 Constanta, Romania*
E-mail: *cambrea.claudia@gmail.com*
[d]*Faculty of Dentistry, 'Ovidius' University, 7 Ilarie Voronca Street, 900 178 Constanta, Romania*
E-mail: *crilucpetcu@gmail.com*

Abstract. Climate change and global warming have been reported to increase spread of foodborne pathogens. To better understand the effects of *Salmonellosis* infections, the objective of this study was to analyse the demographic data of the patients and environmental features from Constanta area, localised on the Black Sea Coast. A retrospective study was conducted on 377 patients with *Salmonella spp.* disease over a 10 years period, between 1st January 2009 and 31st December 2018 at Constanta Clinical Infectious Diseases Hospital from Romania. Demographic (i.e. sex and location) and meteorological (i.e. atmospheric pressure, humidity, atmospheric temperature, and rainfall) data were collected. The results showed that the risk of appearance of *Salmonella* spp. cases during the period June–September comparative with October–May of 2.629 more in condition of increased humidity, and 2.264 more in condition of increased atmospheric temperature. Therefore, our study shows that bacterial pathogens is positively correlated with atmospheric temperature and humidity in Constanta area, as warmer temperatures enable more rapid replication of the pathogen.

Keywords: *Salmonella* spp., environmental factors, temperature, climate.

AIMS AND BACKGROUND

Salmonella represents a major public health concern especially in low socioeconomic areas. *Salmonella* genus is composed of two species, *S. enterica* and *S. bongori*. Further, *S. enterica* is split into six subgroups (i.e. *enterica*, *salamae*,

arizonae, *diarizonae*, *indica*, and *houtenae*) being responsible of more than 95% of human infections. In contrast, *S. bongori* usually infect cold-blooded hosts[1,2].

* For correspondence.

Most of the infections are being developed by serovars of *S. enterica*. The infec- tions namely nontyphoidal *Salmonella* (NTS) can lead often to gastroenteritis. The most spread NTS serovars are represented by Typhimurium and Enteritidis[3]. One recent study shows that each year there are more than 93.8 million human infection of gastroenteritis around the world[4,5]. In this regard, many infections were shown to be influenced by different environmental factors such as climate or land[6]. Many geographical features like climate, weather, and hydrology could influence the frequency transfer of pathogenic microorganisms from environmental medium[7].

The climate change associated with the diseases appearance showed already to comprise approximately 4.6% of all environmental hazards. Furthermore, it has been shown that in 2000 year, the changes in climate produce 2.4% of all diar- rhea in the world and 7% of dengue fever episodes in many industrialised cities[8]. The changes in weather patterns showed to be the reason for leafy vegetables and herbs contamination. Dry climate can lead to dust storms that leave dust particles on vegetables. Interestingly, higher degrees of temperatures can increase the per- centage of microbial growth[9,10]. At the same time, it could negatively influence the insects and pests around farms which could transfer human pathogens to vegetables. Moreover, relative humidity can have a strong effect on human pathogens survival rate[11]. Numerous infections agents and rate of pathogen replication are being more sensitive to climate changes. In this respect, *Salmonella* spp. proliferates more rapidly at higher temperature, especially in animal gut and food. In the areas with lower temperatures and low rainfall restrict transmission of diseases, and climate changes could give the ecological habitat and trigger epidemics[12]. Therefore, changes of climate and environmental factors could contribute to spreading of many foodborne pathogens leading to different infections disease[13].

The present study was undertaken to investigate the effects of *Salmonella* spp. infections and the correlation with environmental factors like atmospheric pres- sure, humidity, atmospheric temperature and rainfall from the Constanta city area.

EXPERIMENTAL

A retrospective study was conducted on 377 patients with *Salmonella* spp. disease over a period of 10 years, between 1st January 2009 and 31st December 2018. All these patients were hospitalised at Constanta Clinical Infectious Diseases Hospital from Romania.

Demographic data (i.e. age, sex, and location) including environmental factors like atmospheric pressure, humidity, atmospheric temperature, and rainfall were collected in the same period. We also analysed if changes in environmental factors influenced evolution of *Salmonella* spp. during the course. Agreement of Ethic Committee from Constanta Clinical Infectious Diseases Hospital from Romania and informed consent of patients and parents were obtained.

During the year, there are four seasons. Spring months were between March and May with an average atmospheric temperature of 15°C. Summer, starting from June to August, has an atmospheric temperature average of 27°C. Autumn starts from September until November, in which the temperatures could be also raised around 18.5°C. In order to control seasonal effects, data were collected from two time periods, the first being based on June till September period, and the other from October till May period. For comparison in the summer period was included the first month of the autumn. June and September months although there are months in different seasons they presented the same average temperature and case number. Average temperature (°C), atmospheric pressure (mb), average humidity (%), and average rainfall amount (mm) on days were measured according with Constanta Monthly Climate Averages[14].

Statistical analysis. For statistical analysis we performed a logistic regression (method Backward Stepwise – Wald) using SPSS Statistics 23. For temperatures, pressure, humidity, and rainfall, the averages of values were calculated for each month over a 10 years period, grouped into two classes: values above and below the monthly average. The new variables obtained and demographic data of the patients were introduced into logistic regression model, and they were considered variable of interest.

The logistic regression equation is:

$$\text{logit}(p) = -0.932 + 0.966\ \text{humidity} + 0.816\ \text{temperature} - 0.380\ \text{sex},$$

where p is the probability of presence of the characteristic of interest. Logit (p) can be back-transformed to p by the following formula:

$$p = 1/(1 + e^{-\text{logit}(p)}).$$

RESULTS AND DISCUSSION

In a study of over 10 years period were admitted in the hospital 377 cases of *Sal- monella* spp. aged between 9 months and 86 years. Children less than 5 years old represent 38.99% from the total cases.

The highest number of cases were noticed in year 2015 (45 cases, 11.93%) in the October-May period and the lowest incidence was seen for the June-September period in 2009 year (9 cases, 2.38%) (Fig. 1).

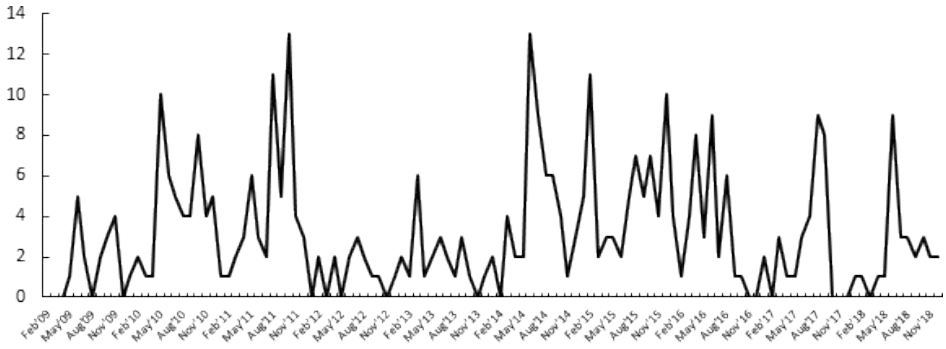


Fig. 1. Evolution of *Salmonella* spp. in Constanta area over 10 years studied period

Moreover, from the total of the patients, 196 (51.98%) were males and 181 (48.01%) females (Fig. 2).

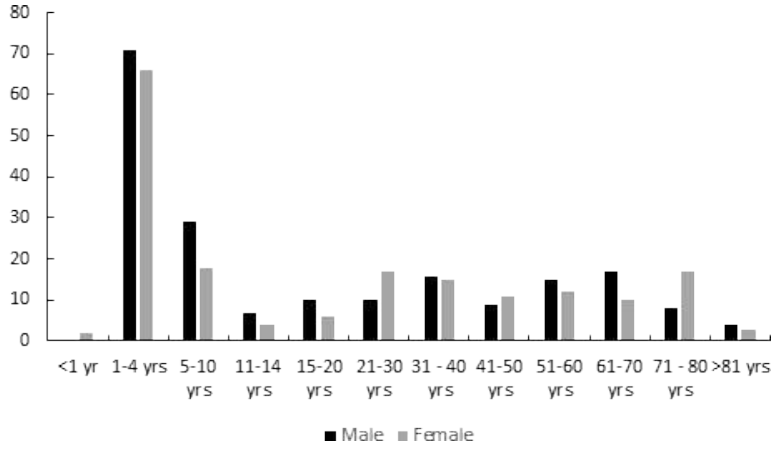


Fig. 2. Distribution between males and females diagnosed with *Salmonellosis*

The higher incidence of locations shows the higher number of patients (231 cases, 61.27%) from urban area comparing with rural area with only 146 cases (38.72%) from all years taken into the study (Fig. 3).

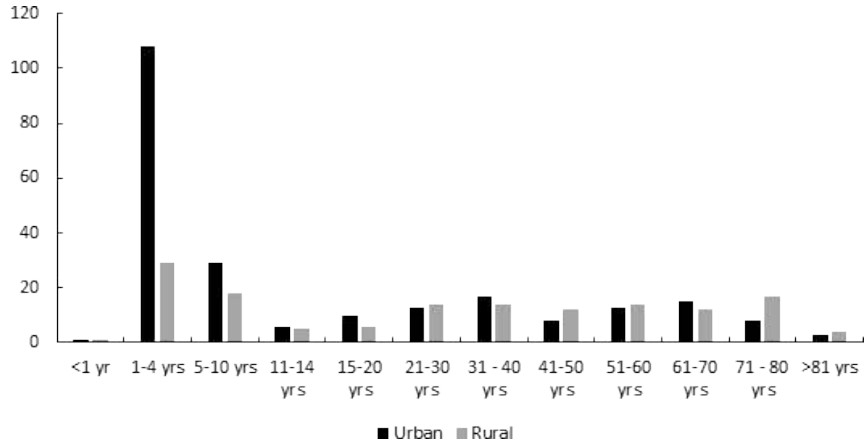


Fig. 3. Distribution of *Salmonella* spp. by local area and group of age

After the distribution by sex and year, and sex and month it was shown that the higher prevalence was for male (196 cases, 51.98%). In June–September were noticed 87 cases of male patients from the total of 178 registered in all years in the mentioned months of the studied years (Figs 4 and 5).

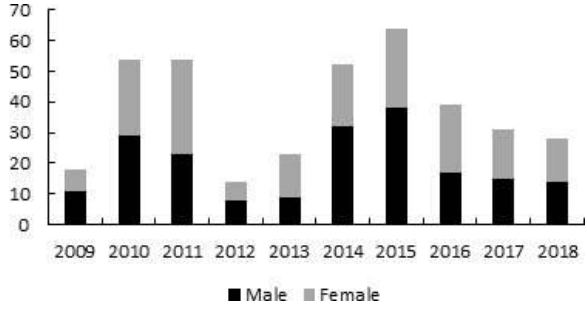


Fig. 4. Repartition by sex and year

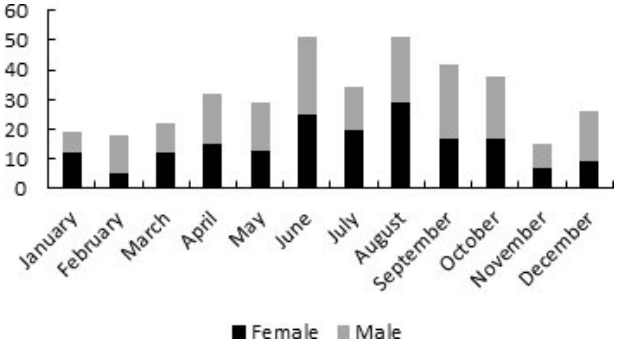


Fig. 5. Repartition by sex and month

From the total cases of Salmonella infections, sepsis was registered in 17 cases while in the other 360 cases were noticed enterocolitis with Salmonella. All the cases from our study were with *Salmonella enterica*, except one case with *Salmonella typhi* in a seafarer.

Over the ten years studied, the medium atmospheric pressure for October–May period was 1017.55 mb, and for June–September period was 1012.86 mb.

Regarding medium temperature over the studied period, we noticed a value of 9.85°C for October–May period and 24.67°C for June–September period.

After measuring the rainfall values, we noticed that medium value for Octo- ber–May period was 56.66 mm, while medium value for June–September period was 39.89 mm.

The medium value for humidity, over the 10 years studied, was for October– May period of 75.2%, while the medium value for June–September period was 62.3%.

When we performed statistical analysis of the prediction variables originally in- cluded in the regression model, the following variables were removed: atmospheric pressuse (p = 0.388), rainfall (p = 0.956), location (p = 0.867), and sex (p = 0.08) being considered variables that do not make a significant contribution ($p > 0.05$). The variable that were included in the model were humidity ($p < 0.001$) and atmospheric temperature ($p <$ 0.001). The results showed that those 2 predictor variables favourise the risk of appearance of *Salmonella* spp. cases during the period June–September comparative with October–May of 2.629 more in condi- tion of increased humidity, and 2.264 more in condition of increased atmospheric temperature. Increased values were considered when they were high comparative with average value of each month (Table 1).

Table 1.Variables in the equation for logistic regression

Independent variable	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for exp (B)	
							lower	upper
Humidity (over average)	0.966	0.224	18.547	1	0.000	2.629	0.447	1.047
Temperature (over average)	0.817	0.232	12.447	1	0.000	2.264	1.438	3.566
Sex	–0.380	0.217	3.060	1	0.080	0.684	0.447	1.047
Constant	–0.932	0.248	14.156	1	0.000	0.394		

In the current study, the effects of climate variation on *Salmonella* spp. infec- tions were examined. Results indicated that atmospheric temperature and humidity are positively correlated with *Salmonella* spp. infections.

One study found no other significant changes in temperature and precipita- tion values. The highest temperature was recorded in 2007 having the average

of 64.95 F with the lowest precipitation (42.33 inches), and in 2009 the highest precipitation of 68.64 inches[15].

The higher prevalence of *Salmonella* spp. infection is found in countries such as Africa, being the most common bacterial isolates in adults and children. The infection was associated with fever together with a 20–25% fatality[16]. However, in developing countries the surveillance of the bacteria is less than 1% (Refs 17 and 18).

Therefore, the higher temperature leads to higher concentrations of bacteria in food industry and can be found especially in warmer months alongside with inadequate cooking of the food. The transmission of the bacteria is made by dif- ferent pathways like direct effect on proliferation or indirect by ingesting food in warm days[19], including the opportunistic infections which are frequently seen in immunosuppressant patients[20,21].

Environmental impact on human health and quality of life is usually related to occupational exposure to chemical substances, dust or particles[22,23]. Environmental contacts and environmental factors like high temperature, high relative humidity and light rainfall are factors that can have a positive influence on Salmonella in- fections and hospitalisations[24,25].

CONCLUSIONS

Salmonella spp. transmission to humans represents a complex process. Higher temperatures combining with eating behaviour could contribute to *Salmonella* spp. infection. However, the environmental factors that influence *Salmonellosis* in the studied period were atmospheric temperature and humidity. Although *Sal- monella* spp. has better adapted at different environmental conditions, the solu- tion for reducing the prevalence of infection will not be easily found. Therefore, increasing the knowledge of environmental transmission can help to minimise the worldwide spreading.

REFERENCES

1. L. L. E. MINOR, M. Y. POPOFF: Designation of *Salmonella enterica* sp. Nov., nom. Rev., as the Type and Only Species of the Genus *Salmonella*: Request for an Opinion. Int J Syst Bacteriol, **37**, 465 (1987).
2. M. W. REEVES, G. M. EVINS, A. A. HEIBA, B. D. PLIKAYTIS, J. J. FARMER II: Clonal Nature of *Salmonella typhi* and Its Genetic Relatdeness to Other Salmonellae as Shown by Multilocus Enzyme Electrophoresis, and Proposal of *Salmonella bongori* comb. Nov J Clin Microbiol, **27**, 313 (1989).
3. T. R. CALLAWAY, T. S. EDRINGTON, R. C. ANDERSON, J. A. BYRD, D. J. NISBET: Gastrointestinal Microbial Ecology and the Safety of Our Food Supply as Related to *Salmonella*. J Anim Sci, **86**, 163 (2007).

4. S. DIACONU, S. C. CAMBREA, L. C. PETCU, S. RUGINĂ: Aspects of Nosocomial Gastroenteritis with Rotavirus in Children Hospitalized in Constanta – Romania. Acta Medica Mediterranea, **33** (Special Issue 2), 1289 (2017).
5. K. HOELZER, A. ISABEL, M. SWITT, M. WIEDMANN: Animal Contact as a Source of Human Non-typhoidal Salmonellosis. Vet Res, **42**, 34 (2011).
6. A. A. LUPU, E. V. IONESCU, M. G. ILIESCU, R. E. ALMASAN, C. OPREA, I. ION, D. M. ILIESCU: Effect of Techirghiol Specific Climate Factors on the Patients Quality of Life with Degenerative Lumbar Pain. J Environ Prot Ecol, **19** (4), 1857 (2018).
7. World Health Organization (WHO): Food Safety Report. 2011. Available at: http://www.who.int/foodsafety/en/, accessed January 25, (2014).
8. V. KENDROVSKI, D. GJORGJEV: Climate Change: Implication for Food-borne Diseases (Salmonella and Food Poisoning among Humans in R. Macedonia). In: Structure and Function of Food Engineering. Rijeka, Croatia, INTECH, 201, 151–170.
9. S. C. CAMBREA, L. C. PETCU, D. M. ILIESCU: Relations of Environmental Factors and Evolution of Boutonneuse Fever in the County of Constanta – Romania. J Environ Prot Ecol, **19** (2), 914 (2018).
10. S. C. CAMBREA, O. C. ARGHIR, A. RASCU, C. L. PETCU: Biochemical Features of an Acute Viral Hepatitis A Outbreak. Revista de Chimie, **69** (6), 1447 (2018).
11. P. R. HUNTER: Climate Change and Waterborne and Vector-borne Disease. J Appl Microbiol, **94** (Suppl), 37S (2003).
12. A. J. McMICHAEL, R. E. WOODRUFF, S. HALES: Climate Change and Human Health: Present and Future Risks. Lancet, **367**, 859 (2006).
13. O. C. ARGHIR, P. M. A. PEREIRA, A. RASCU et al.: The impact of Migrant Tuberculosis on the Chimioresistance Pattern of Antituberculosis Drugs in a Low Burden Tuberculosis European Country. Farmacia, **66** (3), 537 (2018).
14. Constanta Monthly Climate Averages Available at: https://www.worldweatheronline.com/constanta-weather-averages/constanta/ro.aspx.
15. L. AKIL, H. A. AHMAD, R. S. REDDY: Effects of Climate Change on Salmonella Infections. Foodborne Pathogens and Disease. **11** (12), 974 (2014).
16. N. A. FEASEY, G. DOUGAN, R. A. KINGSLEY, R. S. HEYDERMANN, M. A. GORDON: Invasive Non-typhoidal Salmonella Disease: an Emerging and Neglected Tropical Disease in Africa. Lancet, **379**, 2489 (2012).
17. M. TAJKARIMIA, S. A. IBRAHIMB, A. M. FRASERC: Food Safety Challenges Associated with Traditional Foods in Arabic Speaking Countries of the Middle East. Trends Food Sci Technol, **29**, 116 (2013).
18. V. COJOCARU, E. DANTES, M. G. NOVAC et al.: The Value of Adenosine Deaminase Enzime Level in the Positive Diagnosis of Tuberculous Pleural Effusion. Rev de Chimie (Bucharest), **69** (12), 3688 (2018).
19. V. K. JUNEJA, M. V. MELENDRES, L. HUAN et al.: Modeling the Effect of Temperature on Growth of Salmonella in Chicken. Food Microbiol, **24**, 328 (2007).
20. S. C. CAMBREA, G. RESUL, I. BULBUC, M. CAMBREA, F. VASILESCU: Pulmonary Inflammatory Myofibroblastic Tumor in an AIDS Patient. Rom J Morphol Embryol, **55** (2), 407 (2014).
21. E. DANTES, D. E. TOFOLEAN, A. P. FILDAN et al.: Lethal Disseminated Tuberculosis in Patients under Biological Treatment – Two Clinical Cases and a Short Review. Journal of International Medical Research, **46** (7), 2961 (2018).
22. O. C. ARGHIR, E. DANTES, E. BORGAZI, A. RASCU, A. SOCACI: Focus on Asbestos Related Diseases in a Pulmonology Department. Revista de Chimie, **69** (5), 1220 (2018).

23. M. R. OTELEA, O.C. ARGHIR, C. ZUGRAVU, E. NAGHI, S. ANTONIU, A. RASCU. Lung Function and Quality of Life in Workers with Chemical and Dust Exposure. Revista de Chimie, **69** (2), 346 (2018).
24. C. J. CHEN, F. T. WU, C. A. HSIUNG et al.: Risk Factors for Salmonella Gastroenteritis in Children Less than Five Years of Age in Taiwan. The Pediatric Infectious Diseases Journal, **31** (12), e239 (2012).
25. P. WANG, W. B. GOGGINS, E. Y. Y. CHAN: Associations of Salmonella Hospitalisations with Ambient Temperature, Humidity and Rainfall in Hong Kong. Environ Int, **120** (11), 223 (2018).

Received 3 July 2019
Revised 26 July 2019