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Charakterystyka zapaleń wątroby w przebiegu pierwotnych zakażeń herpeswirusowych (wirusem Epsteina-Barr oraz wirusem cytomegalii) u dzieci

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa 2026 r.

Słowa kluczowe: zapalenie wątroby, wirus Epsteina-Barr, wirus cytomegalii, dzieci

Key words: hepatitis, Epstein-Barr Virus, cytomegalovirus, children

Podziękowania

Pragnę podziękować moim Rodzicom za wiarę w moje możliwości. Wasza miłość i mądrość były dla mnie fundamentem, na którym budowałam swoje marzenia.

Dziękuję mojemu Mężowi za wyrozumiałość, siłę i obecność w chwilach zwątpienia. Twoje wsparcie dodaje mi odwagi, aby iść naprzód, nawet gdy droga wydaje się trudna.

Szczególne podziękowania kieruję do moich Dzieci – za Wasz uśmiech, który rozjaśnia każdy dzień, i za nieustanne przypominanie, co w życiu jest naprawdę ważne. Dla Was chcę być przykładem pokazując, że warto dążyć do celu z pasją i determinacją.

Wykaz publikacji stanowiących rozprawę doktorską

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   DOI: https://doi.org/10.5114/ceh.2025.154284
   [IF 1,7, MNiSW 20]

Łączny Impact Factor cyklu publikacji: 8,4

Łączna punktacja Ministerstwa Nauki i Szkolnictwa Wyższego: 360

Spis treści:

Spis tabel i rycin:

Wykaz stosowanych skrótów (wg kolejności alfabetycznej)

AAC – bezkamicze zapalenie pęcherzyka żółciowego (ang. *Acute Acalculous Cholecystitis*)

ALT – aminotransferaza alaninowa (ang. *Alanine Aminotransferase*)

AST – aminotransferaza asparaginianowa (ang. *Aspartate Aminotransferase*)

CLIA – chemiluminescencyjny test immunologiczny (ang. *Chemiluminescent Immunoassay*)

CMV – wirus cytomegalii (ang. *Cytomegalovirus*)

COVID-19 – choroba koronawirusowa 2019 (ang. *Coronavirus Disease 2019*)

CRP – białko C-reaktywne (ang. *C-reactive protein*)

EBV – wirus Epsteina-Barr (ang. *Epstein-Barr virus*)

γ-GT – gamma-glutamylotransferaza (ang. *Gamma-Glutamyl Transferase*)

GGN – górna granica normy

GGTP – gamma-glutamylotranspeptydaza

GBWT – pogrubienie ściany pęcherzyka żółciowego (ang. *gallbladder wall thickening*)

HAV – wirus zapalenia wątroby typu A (ang. *Hepatitis A virus*)

HBV – wirus zapalenia wątroby typu B (ang. *Hepatitis B virus*)

HCV – wirus zapalenia wątroby typu C (ang. *Hepatitis C virus*)

HHV-4 – ludzki herpeswirus 4 (ang. *Human Herpesvirus 4*)

HIV – ludzki wirus niedoboru odporności (ang. *Human Immunodeficiency Virus*)

IM – ang. *infectious mononucleosis*

LDH – dehydrogenaza mleczanowa (ang. *lactate dehydrogenase*)

SD – odchylenie standardowe (ang. *standard deviation*)

ULN – ang. *upper limit of normal*

VCA – antygen kapsydu wirusa (ang. *viral capsid antigen*)

WBC – krwinki białe (ang. *white blood cells*)

Streszczenie w języku polskim

Charakterystyka zapaleń wątroby w przebiegu pierwotnych zakażeń herpeswirusowych (wirusem Epsteina-Barr oraz wirusem cytomegalii) u dzieci

Wstęp

Mononukleoza zakaźna jest powszechnie występującą chorobą będącą efektem pierwotnego zakażenia wirusem Epsteina-Barr (EBV, *Human Gamma-Herpesvirus 4* – HHV-4), który jest patogenem wyłącznie ludzkim. Serologiczne cechy przebytej infekcji stwierdza się u ponad 95% dorosłej populacji na świecie, ale większość osób przechodzi zakażenie w sposób bezobjawowy. Zespół objawów podobnych do obserwowanych w mononukleozie zakaźnej może być wywołany również przez wirus cytomegalii (CMV, *Cytomegalovirus*) także należący do rodziny *Herpesviridae*. W przebiegu pierwotnej infekcji EBV i/lub CMV może rozwinąć się zapalenie wątroby (zgodnie z dostępnymi danymi nawet u 80 – 90% dorosłych z mononukleozą zakaźną) – najczęściej o łagodnym do umiarkowanego przebiegu, choć obserwowane są także objawy kliniczne i laboratoryjne cholestazy oraz bezkamicze zapalenie pęcherzyka żółciowego. Dane literaturowe dotyczące zapalenia wątroby w pierwotnym zakażeniu EBV u dzieci są znacznie bardziej ograniczone niż te odnoszące się do populacji dorosłych. Patogeneza uszkodzenia hepatocytów w zakażeniach EBV i CMV nie jest w pełni wyjaśniona. Oba wirusy nie wykazują tropizmu do komórek wątrobowych ani nabłonka dróg żółciowych. Przypuszczalnie kluczową rolę odgrywa odpowiedź układu odpornościowego indukująca procesy zapalne w wątrobie. W praktyce klinicznej leczenie mononukleozy zakaźnej pozostaje objawowe. Dotychczas nie potwierdzono korzyści ze stosowania acyklowiru, natomiast rola glikokortykosteroidów ogranicza się do wybranych powikłań (np. obturacja dróg oddechowych).

Cele rozprawy doktorskiej

Cele rozprawy doktorskiej były następujące:

1. Analiza przebiegu klinicznego zapalenia wątroby u dzieci z pierwotnym zakażeniem EBV, z uwzględnieniem znaczenia oceny laboratoryjnej funkcji wątroby oraz badania ultrasonograficznego (USG) jamy brzusznej (**Publikacja nr 1.**).

2. Porównanie przebiegu zapalenia wątroby u dzieci z pierwotną infekcją EBV leczonych glikokortykosteroidami (stosowanymi w leczeniu pozawątrobowych powikłań mononukleozy zakaźnej) z grupą pacjentów nieotrzymujących glikokortykosteroidów (**Publikacja nr 2.**).
3. Porównanie przebiegu zapalenia wątroby u dzieci z pierwotnym zakażeniem EBV oraz u dzieci z koinfekcją EBV/ CMV potwierdzoną badaniem serologicznym (**Publikacja nr 3.**).

Materiał i metody

Rozprawa doktorska składa się z cyklu trzech pełnotekstowych publikacji:

1. Badania obserwacyjnego, w którym analizowano przebieg kliniczny zapalenia wątroby u dzieci z pierwotnym zakażeniem EBV (ze szczególnym uwzględnieniem dynamiki zmian aktywności aminotransferaz wątrobowych, częstości występowania cholestatycznego fenotypu choroby, analizy zmian uwidocznionych w badaniu USG) – **Publikacja nr 1.**
2. Badania kliniczno-kontrolnego oceniającego wpływ steroidoterapii stosowanej z powodu pozawątrobowych powikłań mononukleozy zakaźnej na przebieg zapalenia wątroby w pierwotnej infekcji EBV – **Publikacja nr 2.**
3. Badania kliniczno-kontrolnego porównującego przebieg zapalenia wątroby u dzieci z monoinfekcją EBV i pacjentów z koinfekcją EBV/ CMV potwierdzoną badaniem serologicznym – **Publikacja nr 3.**

Wyniki

W **Publikacji nr 1.** wykazano, że częstość występowania objawów związanych z patologią wątroby i dróg żółciowych u dzieci z pierwotnym zakażeniem EBV wynosiła odpowiednio: hepatomegalia – 80,72%, ból brzucha – 31,32%, nudności/ wymioty - 27,11%, żółtaczka – 11,44%, świąd skóry – 11,44%, tkliwość palpacyjna brzucha – 10,84%, ściemnienie moczu – 4,82%, odbarwienie stolca – 1,20%. Podwyższoną aktywność aminotransferazy alaninowej (ALT) i asparaginianowej (AST) obserwowano od pierwszego do trzeciego tygodnia choroby z tendencją do normalizacji od czwartego tygodnia. Aktywność ALT przekraczała pięciokrotność wartości górnej granicy normy (GGN) dla płci i wieku, najczęściej w pierwszym tygodniu choroby u 44/95 (46,31%) pacjentów. Aktywność AST przekraczała pięciokrotność wartości GGN najczęściej w drugim tygodniu choroby u 33/108 (30,55%) dzieci. Aktywność ALT utrzymywała się powyżej normy przez 3 tygodnie, zaś aktywność AST wykazywała dwa szczyty: w pierwszym tygodniu (średnio 186,34 ± 148,17 IU/l) oraz w trzecim tygodniu (średnio 169,00 ± 140,98 IU/l). Aktywność gamma-

glutamylotranspeptydazy (GGTP) była podwyższona w pierwszych trzech tygodniach choroby osiągając najwyższe wartości w drugim tygodniu obserwacji (średnio 183,84 ± 97,48 IU/l). U 60,53% pacjentów występowało podwyższone stężenie bilirubiny całkowitej. Stężenie bilirubiny związanej przekraczało 20% stężenia bilirubiny całkowitej u 18 (47,37%) dzieci, w każdym przypadku wiązało się to z podwyższoną aktywnością GGTP. U 18/166 (10,84%) pacjentów z zapaleniem wątroby obserwowano kliniczne i laboratoryjne objawy cholestazy, dwanaścioro (66,66%) z nich było starszych niż 15 lat. U wszystkich pacjentów z objawami cholestazy występowały ultrasonograficzne cechy bezkamiczego zapalenia pęcherzyka żółciowego.

W **Publikacji nr 2.** zidentyfikowano istotne różnice pomiędzy badanymi grupami dotyczące: czasu trwania hospitalizacji, który był dłuższy w grupie pacjentów leczonych glikokortykosteroidami: 5,0 ± 2,1 dni vs. 4,3 ± 2,3 dni dla grupy nieotrzymującej glikokortykosteroidów (p = 0,015); częstości występowania klasycznych objawów mononukleozy zakaźnej, które były częstsze u pacjentów leczonych glikokortykosteroidami: 84,61% vs. 61,29% w grupie 2 (p = 0,000); występowania gorączki, którą częściej obserwowano u pacjentów leczonych glikokortykosteroidami: 90,38% vs. 79,03% w grupie drugiej (p = 0,040); czasu trwania gorączki, która utrzymywała się dłużej u pacjentów zakwalifikowanych do leczenia glikokortykosteroidami: 7,5 ± 4,7 dni vs. 5,9 ± 4,8 dni u dzieci nieotrzymujących glikokortykosteroidów (p = 0,049); częstości występowania powiększenia wątroby w badaniu palpacyjnym brzucha, co stwierdzano u 85,58% dzieci z grupy pierwszej vs. 72,58% z grupy drugiej (p = 0,040); podobnie powiększenie śledziony częściej stwierdzano u dzieci otrzymujących glikokortykostaroidy: 73,08% vs. 56,45% w grupie 2 (p = 0,027). Istotne różnice w zakresie wyników badań laboratoryjnych dotyczyły: średniej aktywności ALT w pierwszym tygodniu choroby, która była wyższa u dzieci nieotrzymujących glikokortykosteroidów: 288,82 ± 170,16 IU/l (50,00 – 725,00) vs. 205,34 ± 115,40 IU/l (39,00 – 516,00) w grupie pierwszej (p = 0,024); średniej aktywności AST, która była w obu grupach najwyższa w pierwszym i trzecim tygodniu choroby, jednak różnice te były istotne statystycznie jedynie w grupie dzieci nieleczonych glikokortykosteroidami (p = 0,024); średnia aktywność AST istotnie różniła się pomiędzy grupami w pierwszym i trzecim tygodniu choroby: w 1. tygodniu wynosiła 170,63 ± 159,47 IU/l (40,00 – 852,00) vs. 218,85 ± 128,22 IU/l (42,00 – 589,00) odpowiednio w grupie 1. i 2. (p = 0,009); zaś w 3. tygodniu choroby wynosiła 151,09 ± 138,57 IU/l (31,00 – 578,00) vs. 235,50 ± 170.27 IU/l (67,00 – 617,00) odpowiednio w grupie 1. i 2. (p = 0,016).

W **Publikacji nr 3.** wykazano statystycznie istotne różnice w przebiegu zapalenia wątroby pomiędzy dziećmi z potwierdzoną serologicznie pierwotną infekcją EBV (grupa 1.) a

pacjentami z potwierdzoną serologicznie koinfekcją EBV/ CMV (grupa 2.). Dotyczyły one częstości występowania poszczególnych objawów choroby: zapalenie gardła i migdałków podniebiennych częściej obserwowano w pierwszej grupie - u 148 (90,24%) vs. 65 (80,25%) w grupie drugiej (p = 0,047); wymioty/ nudności częściej występowały w grupie 2. – 35 (43,21%) vs. 45 (27,44%) w grupie 1., p = 0,010 (OR = 2,01 [95% CI: 1,15 – 3,51]); ból brzucha zgłaszało 37 (45,67%) dzieci z drugiej grupy vs. 52 (31,71%) dzieci z pierwszej grupy, p = 0,042 (OR = 1,81 [95% CI: 1,05 – 3,13]); ściemnienie moczu obserwowano częściej u pacjentów z grupy 2. – 12 (14,81%) niż w grupie 1. – 8 (4,88%), p = 0,006 (OR = 3,39 [95% CI: 1,33 – 8,67]). W zakresie diagnostyki laboratoryjnej i obrazowej istotne różnice dotyczyły: stężenia CRP, które było wyższe u pacjentów z pierwszej grupy – 21,93 ± 16,54 mg/dl niż w grupie drugiej – 15,15 ± 12,85 mg/dl (p = 0,001); odsetka granulocytów obojętnochłonnych w rozmazie ręcznym krwinek białych, który był wyższy w pierwszej grupie – 32,07 ± 13,25% niż w drugiej – 25,33% ± 12,35% (p = 0,000); częstości uzyskiwania nieprawidłowych wyników badania USG jamy brzusznej, co częściej zdarzało się w grupie 2. (u 100% badanych) niż w grupie 1. (66,46%), p = 0,045. Kryteria cięższego przebiegu choroby (stworzone na potrzeby niniejszego badania) istotnie częściej spełniały dzieci z grupy 2. – 60 (74,07%) vs. 96 (58,54%) w grupie 1., p = 0,018 (OR = 2,02 [95% CI: 1,13 – 3,64]).

Wnioski

1. U dzieci z mononukleozą zakaźną ocena aktywności aminotransferaz: ALT, AST, GGTP oraz USG jamy brzusznej mają istotne znaczenie w kontekście zidentyfikowania pacjentów z cięższym, cholestatycznym przebiegiem zapalenia wątroby lub bezkamiczym zapaleniem pęcherzyka żółciowego, którzy wymagają wnikliwej kontroli po ustąpieniu objawów choroby (**Publikacja nr 1.**).
2. Steroidoterapia (najczęściej stosowana celem leczenia obturacji dróg oddechowych w przebiegu mononukleozy zakaźnej) może łagodzić przebieg zapalenia wątroby w pierwotnej infekcji EBV u dzieci (zwłaszcza w pierwszym tygodniu choroby), jednak nie wpływa na wartości parametrów cholestazy i częstość występowania nieprawidłowości dotyczących wątroby i dróg żółciowych uwidacznianych w USG (nie modyfikuje ryzyka rozwoju powikłań). Wczesne obniżenie aktywności ALT i AST u dzieci leczonych steroidami może maskować rzeczywistą dynamikę choroby, dlatego kluczowa jest kontrola aktywności tych enzymów po zakończeniu steroidoterapii (**Publikacja nr 2.**).

3. U dzieci z zapaleniem wątroby w przebiegu pierwotnego zakażenia EBV potwierdzona serologicznie koinfekcja CMV wiązała się z większym nasileniem objawów cholestazy i częstszym występowaniem nieprawidłowości dotyczących wątroby i dróg żółciowych w USG. U tych pacjentów obserwowano też cięższy przebieg zapalenia wątroby (wyższa aktywność ALT i AST) w początkowej fazie choroby (**Publikacja nr 3.**).

Streszczenie w języku angielskim

Characteristics of hepatitis in the course of primary herpesvirus infections (Epstein-Barr virus and Cytomegalovirus) in children

Introduction

Infectious mononucleosis (IM) is a common disease resulting from primary infection with the Epstein–Barr Virus (EBV, *Human Gamma-Herpesvirus 4* – HHV-4), which is a human-exclusive pathogen. Serological markers of past infection are found in more than 95% of adults worldwide, although the majority of individuals experience the infection asymptomatically. A clinical syndrome resembling IM may also be caused by cytomegalovirus (CMV), which likewise belongs to the *Herpesviridae* family. During primary EBV and/or CMV infection, hepatitis may develop (according to available data) in as many as 80–90% of adults with IM. The disease course is usually mild to moderate, although clinical and laboratory features of cholestasis as well as acalculous cholecystitis (AAC, *Acute Acalculous Cholecystitis*) have also been described. Literature data on hepatitis in children with primary EBV infection are limited compared with the adult population. The pathogenesis of hepatocellular injury in EBV and CMV infections remains incompletely understood. Neither of the viruses exhibits tropism for hepatocytes or biliary epithelium. The immune response triggered by the infection is presumed to play a key role in inducing inflammatory processes in the liver. In clinical practice, the treatment of IM is supportive. To date, the benefits of acyclovir therapy have not been confirmed, and the role of glucocorticoids remains limited to selected complications (e.g., airway obstruction).

The aims of the doctoral dissertation

The objectives of this dissertation were as follows:

1. To analyse the clinical course of hepatitis in children with primary EBV infection, taking into account laboratory assessment of liver function and abdominal ultrasonography (USG) (**Publication No. 1**).
2. To compare the course of hepatitis in children with primary EBV infection who were

treated with glucocorticoids (administered for extrahepatic complications of IM) with patients not receiving glucocorticoids (**Publication No. 2**).

3. To compare the course of hepatitis in children with primary EBV infection and those with serologically confirmed EBV/CMV coinfection (**Publication No. 3**).

Material and Methods

The dissertation consists of a series of three full-text publications:

1. An observational study analysing the clinical course of hepatitis in children with primary EBV infection, with particular focus on temporal changes in liver aminotransferase activity, the incidence of cholestatic disease phenotype, and abnormalities identified on abdominal ultrasonography (**Publication No. 1**).
2. A case – control clinical study assessing the impact of steroid therapy**,** administered for the treatment of extrahepatic complications of IM**,** on the course of hepatitis in primary EBV infection **(Publication No. 2)**.
3. A case – control clinical study comparing the course of hepatitis in children with EBV monoinfection and children with serologically confirmed EBV/CMV coinfection (**Publication No. 3**).

Results

In **Publication No. 1**, the prevalence of liver and biliary tract–related symptoms in children with primary EBV infection was as follows: hepatomegaly – 80.72%, abdominal pain – 31.32%, nausea/vomiting – 27.11%, jaundice – 11.44%, pruritus – 11.44%, abdominal tenderness – 10.84%, dark urine – 4.82%, pale stools – 1.20%. Elevated ALT and AST levels were observed from the first to the third week of illness, with a tendency to normalise from the fourth week onward. ALT values exceeded five times the upper limit of normal (ULN) most frequently during the first week, in 44/95 patients (46.31%). AST exceeded five times ULN most often in the second week, occurring in 33/108 children (30.55%). ALT levels remained elevated for three weeks, whereas AST activity exhibited two peaks: in the first week (mean 186.34 ± 148.17 IU/L) and in the third week (mean 169.00 ± 140.98 IU/L). Gamma-glutamyltransferase (GGT) activity was elevated during the first three weeks, with the highest mean value in the second week (183.84 ± 97.48 IU/L). Elevated total bilirubin concentration was found in 60.53% of patients. Conjugated bilirubin exceeded 20% of total bilirubin in 18 children (47.37%), and in all cases this finding was associated with elevated GGT activity. In 18/166 (10.84%) patients, clinical and laboratory

features of cholestasis were observed and 12 (66.66%) of them were older than 15 years. All children with cholestasis demonstrated ultrasound features of AAC.

In Publication No. 2, significant differences between groups included: the length of hospitalisation – was longer in glucocorticoid-treated patients: 5.0 ± 2.1 days vs. 4.3 ± 2.3 days, (p = 0.015); the prevalence of classic IM symptoms – occurred more common in glucocorticoid-treated patients: 84.61% vs. 61.29%, (p = 0.000); occurrence of fever – was more frequent in the glucocorticoid group: 90.38% vs. 79.03%, (p = 0.040); the duration of fever – was longer in steroid-treated children: 7.5 ± 4.7 days vs. 5.9 ± 4.8 days, (p = 0.049); hepatomegaly on physical examination was observed more frequently in the steroid-treated group: 85.58% vs. 72.58%, (p = 0.040); splenomegaly was also more frequent in children receiving glucocorticosteroids: 73.08% vs. 56.45%, (p = 0.027). Significant differences in laboratory findings included: mean ALT activity in the first week was significantly higher in children not receiving glucocorticosteroids: 288.82 ± 170.16 IU/L vs. 205.34 ± 115.40 IU/L, (p = 0.024); mean AST activity, which peaked in the first and third weeks in both groups, with statistically significant differences observed only in children not treated with glucocorticosteroids (p = 0.024). Between-group differences in AST activity were significant in the first week: 170.63 ± 159.47 IU/L (40,00 – 852,00) vs. 218.85 ± 128.22 IU/L (42,00 – 589,00), p = 0.009 and the third week: 151.09 ± 138.57 IU/L (31,00 – 578,00) vs. 235.50 ± 170.27 IU/L (67,00 – 617,00), p = 0.016.

In **Publication No. 3**, statistically significant differences in the course of hepatitis were demonstrated between children with serologically confirmed primary EBV infection (Group 1) and patients with serological evidence of primary EBV and CMV coinfection (Group 2). Regarding clinical manifestations: pharyngitis/tonsillitis was more frequent in Group 1: 148 (90.24%) vs. 65 (80.25%), p = 0.047; nausea/vomiting occurred more often in Group 2: 35 (43.21%) vs. 45 (27.44%), p = 0.010; OR = 2.01 [95% CI: 1.15–3.51]; abdominal pain was reported more frequently in Group 2: 37 (45.67%) vs. 52 (31.71%), p = 0.042; OR = 1.81 [95% CI: 1.05–3.13]; dark urine was observed more often in Group 2: 12 (14.81%) vs. 8 (4.88%), p = 0.006; OR = 3.39 [95% CI: 1.33–8.67]. Significant differences in laboratory and imaging findings included: higher CRP concentrations in Group 1: 21.93 ± 16.54 mg/dL vs. 15.15 ± 12.85 mg/dL, p = 0.001; a higher percentage of neutrophils in Group 1: 32.07 ± 13.25% vs. 25.33 ± 12.35%, p = 0.000; a higher frequency of abnormal abdominal ultrasound results in Group 2: 100% vs. 66.46%, p = 0.045. The criteria for a more severe course of disease (developed for this study) were met significantly more often by children in Group 2: 60 (74.07%) vs. 96 (58.54%), p = 0.018; OR = 2.02 [95% CI: 1.13–3.64].

Conclusions

1. In children with IM, laboratory assessment of ALT, AST, GGT activity and abdominal ultrasound play an important role in identifying patients with a more severe, cholestatic course of hepatitis or AAC. These patients require careful follow-up after the resolution of clinical symptoms (**Publication No. 1**).
2. Steroid therapy (most commonly used to treat airway obstruction in the course of IM) may alleviate the course of hepatitis during primary EBV infection in children, particularly in the first week of illness. However, it does not affect cholestasis parameters nor the frequency of abnormalities of the liver and biliary tract detected by ultrasound (i.e., it does not modify the risk of complications). Early reductions in ALT and AST activity in children treated with steroids may mask the true dynamics of the disease; therefore, post-treatment monitoring of these enzymes is essential (**Publication No. 2**).
3. In children with hepatitis resulting from the primary EBV infection, serologically confirmed CMV coinfection was associated with more pronounced cholestasis and more frequent liver and biliary tract abnormalities on ultrasound examination. These patients also demonstrated a more severe course of hepatitis (higher ALT and AST activity) in the early phase of the disease (**Publication No. 3**).

Wstęp uzasadniający połączenie wskazanych publikacji w jeden cykl, jak i komentujący osiągnięcie naukowe na tle dotychczasowego stanu wiedzy.

Mononukleoza zakaźna jest powszechnie występującą chorobą będącą efektem pierwotnego zakażenia wirusem Epsteina-Barr (EBV, *Human Gamma-Herpesvirus 4* – HHV-4), który jest patogenem wyłącznie ludzkim. Zakażenie zwykle następuje w dzieciństwie lub w wieku młodzieńczym, głównie poprzez kontakt ze śliną; rzadziej drogą płciową, poprzez transfuzje preparatów krwi lub przeszczepienia narządów. Serologiczne cechy przebytej infekcji stwierdza się u ponad 95% dorosłej populacji na świecie [31,49]. Zwykle zakażenie przebiega bezobjawowo, a ryzyko wystąpienia pełnoobjawowej mononukleozy zakaźnej rośnie z wiekiem [9]. Klasyczna triada objawów mononukleozy zakaźnej obejmuje gorączkę mogącą trwać do kilku tygodni, uogólnioną limfadenopatię oraz zapalenie migdałków podniebiennych. Najczęstsze pozostałe objawy to powiększenie wątroby i śledziony, obrzęk tkanki łącznej nasady nosa (objaw Glanzmanna), mowa nosowa, nieprzyjemny zapach z ust. Często dochodzi do rozwoju obturacji dróg oddechowych w wyniku proliferacji tkanki limfatycznej węzłów chłonnych i pierścienia Waldeyera.

Zespół objawów podobnych do obserwowanych w mononukleozie zakaźnej może być wywołany również przez wirus cytomegalii (CMV, *Cytomegalovirus*), który tak jak EBV należy do rodziny *Herpesviridae* [24]. Pierwotne zakażenie skutkuje latencją wisusów w limfocytach B, monocytach/ makrofagach, komórkach mięśni gładkich, komórkach śródbłonka, neuronach. Reaktywacja może mieć ciężki przebieg u pacjentów z niedoborem odporności, zwłaszcza po przeszczepieniach komórek krwiotwórczych lub narządów litych.

W przebiegu pierwotnej infekcji EBV i/lub CMV może rozwinąć się zapalenie wątroby (zgodnie z dostępnymi danymi występuje ono nawet u 80 – 90% dorosłych z mononukleozą zakaźną) – najczęściej o łagodnym do umiarkowanego przebiegu, choć obserwowane są także objawy kliniczne i laboratoryjne cholestazy oraz bezkamicze zapalenie pęcherzyka żółciowego [28, 46]. Dane literaturowe dotyczące zapalenia wątroby w pierwotnym zakażeniu EBV u dzieci są znacznie bardziej ograniczone niż te odnoszące się do populacji dorosłych.

Patogeneza uszkodzenia hepatocytów w zakażeniach EBV i CMV nie jest w pełni wyjaśniona. Oba wirusy nie wykazują tropizmu do komórek wątrobowych ani nabłonka dróg

żółciowych. Przypuszczalnie kluczową rolę odgrywa odpowiedź układu odpornościowego – m.in. napływ limfocytów T CD8+ zakażonych EBV do miąższu wątroby oraz produkcja mediatorów zapalnych (TNF-α, IFN-γ, Fas ligand), co może wtórnie zaburzać transport żółci w obrębie kanalików żółciowych i zatok sprzyjając cholestatycznemu fenotypowi zapalenia wątroby [26, 55].

Mononukleoza zakaźna zwykle przebiega łagodnie i w sposób samoograniczający się. Możliwy jest cięższy i powikłany przebieg choroby, przy czym dotychczas nie ustalono czynników ryzyka takiego przebiegu, a tym samym sposobu zapobiegania.

W praktyce klinicznej leczenie mononukleozy zakaźnej pozostaje objawowe. Dotychczas nie potwierdzono korzyści ze stosowania acyklowiru, natomiast rola glikokortykosteroidów ogranicza się do wybranych powikłań (np. obturacja dróg oddechowych) [43,55]. Glikokortykosteroidy stosuje się najczęściej w przypadku obturacji dróg oddechowych. Nie ustalono ostatecznie ich wpływu na przebieg zapalenia wątroby w przebiegu mononukleozy zakaźnej u dzieci.

Częstość występowania koinfekcji EBV/ CMV u dzieci nie jest znana, część autorów opisywała cięższy przebieg mononukleozy zakaźnej u tych pacjentów w porównaniu do monoinfekcji EBV [59]. Doniesienia te dotyczyły także nasilenia zapalenia wątroby [23, 34].

Uzasadnienie

Zapalenie wątroby w przebiegu zakażeń wirusami EBV i CMV jest częstym problemem w praktyce pediatrycznej. Dostępne dane literaturowe odnoszące się do problemów poruszanych w powyższych pracach są ograniczone - zwłaszcza w kontekście populacji pediatrycznej.

Głównym obszarem mojej pracy naukowo-badawczej w ramach niniejszej rozprawy jest analiza manifestacji hepatologicznych i determinantów ciężkiego przebiegu zakażeń herpeswirusowych (EBV i CMV) u dzieci.

Cykl publikacji otwiera praca oryginalna (**Rutkowska M, Pokorska-Śpiewak M. Epstein Barr Virus Hepatitis - A Mild Clinical Symptom or a Threat? Vaccines. 2023; 11: 1119, 1-14.**), w której analizowano przebieg zapalenia wątroby u dzieci z pierwotną infekcją EBV. Skupiłam się na przebiegu klinicznym choroby, analizie wyników badań laboratoryjnych (ze szczególnym uwzględnieniem aktywności enzymów wątrobowych) oraz nieprawidłowościach w badaniu ultrasonograficznym jamy brzusznej. Praca stanowi punkt wyjścia dla kolejnych artykułów, w których analizowano szczególne aspekty choroby w wybranych podgrupach pacjentów (otrzymujących glikokortykosteroidy oraz u dzieci z koinfekcją EBV/ CMV).

Kontynuacją opisu populacyjnego zawartego w pierwszej pracy jest kolejna publikacja oryginalna (**Rutkowska M**, **Pokorska-Śpiewak M. The influence of steroid therapy of complications of infectious mononucleosis on the course of Epstein-Barr virus hepatitis. Clin Exp HEPATOL. 2023; 9 (4): 375–385**) oceniająca wpływ krótkotrwałej systemowej terapii glikokortykosteroidami stosowanej z powodu pozawątrobowych powikłań mononukleozy zakaźnej na dynamikę zmian parametrów wątrobowych u dzieci z zapaleniem wątroby w przebiegu pierwotnej infekcji EBV. Wyniki analizy wskazujące na niższą aktywność enzymów wątrobowych na początku choroby u dzieci otrzymujących glikokortykosteroidy są istotne wobec niejednoznacznych zaleceń dotyczących steroidoterapii w mononukleozie zakaźnej.

W kolejnej pracy oryginalnej (**Rutkowska M**, **Pokorska-Śpiewak M. The impact of acute CMV coinfection on the course of EBV hepatitis in children. Clin Exp HEPATOL. 2025; 11, 3: 1–10**) oceniono wpływ modulatora infekcyjnego jakim jest potencjalna koinfekcja CMV na fenotyp zapalenia wątroby w przebiegu pierwotnego zakażenia EBV u dzieci.

Założenia i cele pracy

Główne cele rozprawy doktorskiej, na którą składa się cykl trzech publikacji to:

1. Analiza przebiegu klinicznego zapalenia wątroby u dzieci z pierwotnym zakażeniem EBV, z uwzględnieniem znaczenia oceny laboratoryjnej funkcji wątroby oraz badania USG jamy brzusznej (**Publikacja nr 1.**).
2. Porównanie przebiegu zapalenia wątroby u dzieci z pierwotną infekcją EBV leczonych glikokortykosteroidami (stosowanymi w leczeniu pozawątrobowych powikłań mononukleozy zakaźnej) z grupą pacjentów nieotrzymujących glikokortykosteroidów (**Publikacja nr 2.**).
3. Porównanie przebiegu zapalenia wątroby u dzieci z serologicznie potwierdzonym pierwotnym zakażeniem EBV oraz u dzieci z koinfekcją EBV/ CMV potwierdzoną badaniem serologicznym (**Publikacja nr 3.**).

Materiał i metody

Rozprawa doktorska składa się z trzech pełnotekstowych publikacji będących retrospektywnymi analizami klinicznymi opartymi na dokumentacji medycznej pacjentów (w wieku od 18 miesięcy do 18 lat) Kliniki Chorób Zakaźnych Wieku Dziecięcego Warszawskiego Uniwersytetu Medycznego oraz Wojewódzkiego Szpitala Zakaźnego w Warszawie hospitalizowanych z powodu zapalenia wątroby w przebiegu pierwotnej infekcji EBV. Badania obejmują okres od listopada 2017 r. do maja 2024 r. i koncentrują się na następujących aspektach analizy zapalenia wątroby w przebiegu pierwotnej infekcji EBV:

1. **Publikacja nr 1.** – ogólna charakterystyka zapalenia wątroby w przebiegu pierwotnej infekcji EBV u dzieci (ze szczególnym uwzględnieniem dynamiki zmian aktywności aminotransferaz wątrobowych, częstości występowania cholestatycznego fenotypu choroby, analizy zmian uwidocznionych w badaniu USG jamy brzusznej);
2. **Publikacja nr 2.** – wpływ steroidoterapii stosowanej z powodu pozawątrobowych powikłań mononukleozy zakaźnej na przebieg zapalenia wątroby w pierwotnej infekcji EBV;
3. **Publikacja nr 3.** – porównanie przebiegu zapalenia wątroby u dzieci z monoinfekcją EBV oraz u tych z koinfekcją EBV/ CMV potwierdzonymi badaniami serologicznymi.

Wszystkie analizy opierają się na podobnych **kryteriach włączenia** pacjentów do badania:

1. ostre zakażenie EBV potwierdzone obecnością przeciwciał w klasie IgM przeciwko antygenowi kapsydu wirusa (ang. *viral capsid antygen,* VCA) za pomocą testu opartego na metodzie chemiluminescencji (ang. *chemiluminescence immunoassay,* CLIA);
2. aktywność ALT przekraczająca wartość górnej granicy normy dla płci i wieku [52]

oraz **kryteriach wykluczenia**:

1. potwierdzone inne wirusowe zapalenia wątroby (HAV – wirus zapalenia wątroby typu A, ang. *Hepatitis A Virus;* HBV – wirus zapalenia wątroby typu B, ang. *Hepatitis B Virus;* HCV – wirus zapalenia wątroby typu C, ang. *Hepatitis C Virus*);
2. przewlekłe choroby wątroby;
3. zakażenie ludzkim wirusem niedoboru odporności (ang. *Human Immunodeficiency Virus*, HIV);
4. leki o działaniu potencjalnie hepatotoksycznym;
5. ciężkie choroby przewlekłe zaburzające funkcjonowanie układu odpornościowego.

Analizie poddano parametry kliniczne (czas trwania objawów, powikłania, kryteria ciężkiego przebiegu), laboratoryjne (aktywność aminotransferaz wątrobowych: ALT, AST,

GGTP; stężenie bilirubiny wraz z frakcjami, morfologię krwi, stężenie CRP i prokalcytoniny - PCT), obrazowe (USG jamy brzusznej, w którym szczególną uwagę zwracano na hepatosplenomegalię oraz zmiany w obrębie dróg żółciowych i pęcherzyka żółciowego) oraz przebieg hospitalizacji.

W publikacji nr 3. w obu badanych grupach zidentyfikowano pacjentów spełniających **kryteria cięższego przebiegu choroby** (stworzone na potrzeby niniejszego badania), które obejmowały:

1. występowanie trzech lub więcej spośród następujących objawów: nudności/ wymioty, ściemnienie moczu (kolor moczu wyraźnie ciemniejszy niż w stanie zdrowia), odbarwienie stolca, żółtaczka, świąd skóry, ból brzucha lub
2. minimum jeden nieprawidłowy wynik spośród poniższych badań laboratoryjnych:
   - aktywność ALT równa lub wyższa niż pięciokrotność GGN dla płci i wieku [52],
   - podwyższona aktywność GGTP w surowicy,
   - stężenie bilirubiny bezpośredniej wyższe lub równe 20% stężenia bilirubiny całkowitej lub
3. cechy zapalenia pęcherzyka żółciowego lub dróg żółciowych w USG (rozdęcie pęcherzyka żółciowego, pogrubienie ściany pęcherzyka > 3 mm, błotko żółciowe, obecność płynu wokół pęcherzyka żółciowego).

Do analizy ststystycznej wykorzystano licencjonowany program STATISTICA 13.3 stosując testy nieparametryczne (Kruskal-Wallis, Mann-Whitney U) i testy dla zmiennych jakościowych (χ^2, Fishera), przy poziomie istotności $p < 0,05$.

Porównanie kluczowych elementów metodologii publikacji stanowiących podstawę niniejszej rozprawy przedstawia tabela 1.

Tabela. 1. Porównanie kluczowych elementów metodologii trzech retrospektywnych analiz klinicznych, na których oparta jest rozprawa doktorska.

Tytuł publikacji	***EBV Hepatitis – A Mild Clinical Symptom or a Threat?***	***The Influence of Steroid Therapy on the Course of EBV Hepatitis.***	***The Impact of Acute CMV Coinfection on the Course of EBV Hepatitis in Children.***
Okres obserwacji	08.2017 – 03.2023	08.2017 – 03.2023	11.2017 – 05.2024
Liczebność badanych grup	166 dzieci	166 dzieci: Grupa 1: 104 (62,65%) - pacjenci leczeni steroidami; Grupa 2: 62 (37,35%) - pacjenci nieotrzymujący steroidów.	244 dzieci: Grupa 1: 164 (67,22%) - pacjenci z pozytywnymi IgM dla VCA EBV; Grupa 2: 80 (32,78%) - pacjenci z pozytywnymi IgM dla EBV i CMV.
Kryteria włączenia	1. Pozytywny wynik oznaczenia przeciwciał w klasie IgM anty-VCA EBV. 2. Aktywność ALT przekraczająca wartość górnej granicy normy dla płci i wieku [52].		
Kryteria wykluczenia	1. Pozytywny wynik oznaczenia przeciwciał w klasie IgM dla CMV. 2. Zakażenia: HAV, HBV, HCV, HIV. 3. Przewlekłe choroby wątroby. 4. Przyjmowanie leków hepatotoksycznych. 5. Choroby przewlekłe upośledzające odporność.		1. Zakażenia: HAV, HBV, HCV, HIV. 2. Przewlekłe choroby wątroby. 3. Przyjmowanie leków hepatotoksycznych. 4. Choroby przewlekłe upośledzające odporność.
Główne analizowane zmienne	Aktywność ALT, AST, GGTP; stężenie bilirubiny z frakcjami, CRP; morfologia krwi; USG jamy brzusznej.		Aktywność ALT, AST, GGTP; stężenie bilirubiny z frakcjami, CRP; morfologia krwi; USG jamy brzusznej;

			objawy cholestazy; dodatkowe kryteria ciężkiego przebiegu choroby.
Metody statystyczne	Test Kruskala – Wallisa	Mann–Whitney U Test, Test Kruskala –Wallisa, test χ^2, test Fishera	

Skróty: ALT – aminotransferaza alaninowa, AST – aminotransferaza asparaginianowa, CMV – wirus cytomegalii, CRP – białko C-reaktywne, EBV – wirus Epsteina-Barr, GGTP – gamma-glutamylotranspeptydaza, HAV – wirus zapalenia wątroby typu A, HBV – wirus zapalenia wątroby typu B, HCV – wirus zapalenia wątroby typu C, HIV – ludzki wirus niedoboru odporności, VCA – antygen kapsydu wirusa (ang. *viral capsid antigen*).

Kopie opublikowanych prac

Publikacja nr 1.

Rutkowska M, Pokorska-Śpiewak M.

Epstein Barr Virus Hepatitis – A Mild Clinical Symptom or a Threat?

Vaccines. 2023; 11: 1119: 1-14.

vaccines

MDPI

Article

Epstein Barr Virus Hepatitis—A Mild Clinical Symptom or a Threat?

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Abstract: The present study aimed to characterize pediatric patients diagnosed with hepatitis associated with primary Epstein-Barr Virus (EBV) infection. We described the changes in liver aminotransferases activity during the disease, and we analyzed the results of abdominal ultrasonography. A retrospective study was performed by analyzing the medical records of 166 immunocompetent children diagnosed with primary EBV hepatitis hospitalized at the Department of Children's Infectious Diseases, Medical University of Warsaw, Regional Hospital of Infectious Diseases in Warsaw, between August 2017 and March 2023. Elevated alanine aminotransferase (ALT) activity was noted in the first three weeks of the disease. In 46.3% of patients, ALT values exceeded five times the upper limit of the laboratory norm in the first week of illness. Aspartate aminotransferase activity increased from the first to fourth week from the onset of symptoms and showed two peaks in the first and third weeks. The changes over time of mean AST activity demonstrated significance. Transient cholestatic liver disease was the predominant type of hepatic involvement in 10.8% of children; 66.6% of them were older than 15 years. Clinical and ultrasound criteria of acute acalculous cholecystitis (AAC) were met in three female patients over 16 years of age. Hepatitis associated with primary EBV infection is usually a mild and self-limiting condition. Significantly elevated values of liver enzymes with features of cholestatic liver disease may occur in patients with a more severe course of the infection.

Keywords: Epstein-Barr virus; alanine aminotransferase; aspartate aminotransferase; children; adolescents; viral hepatitis

Citation: Rutkowska, M.; Pokorska-Śpiewak, M. Epstein Barr Virus Hepatitis—A Mild Clinical Symptom or a Threat? *Vaccines* **2023**, *11*, 1119. https://doi.org/10.3390/vaccines11061119

Academic Editor: Yee-Joo Tan

Received: 11 May 2023
Revised: 12 June 2023
Accepted: 16 June 2023
Published: 19 June 2023

1. Introduction

The Epstein-Barr virus (EBV, HHV-4, human gamma-herpesvirus 4) is a double-stranded DNA virus. EBV causes approximately 90% of infectious mononucleosis (IM), so-called glandular fever, which typically manifests itself as the classic triad of symptoms: fever, pharyngitis, and lymphadenopathy. The remaining 10% of IM cases are caused by: cytomegalovirus (CMV), human herpes virus 6, herpes simplex virus, and human immunodeficiency virus (HIV) [1]. EBV infection can take various clinical forms, as the virus can affect virtually any organ. EBV is an etiologic agent of other disorders as well, for example, Burkitt lymphoma, nasopharyngeal carcinoma, undifferentiated B- or T-lymphocyte lymphomas, leiomyosarcoma, X-linked lymphoproliferative syndrome, and post-transplantation lymphoproliferative disorders. EBV is known to cause 5.6% of infection-associated cancers [2]. EBV infection is estimated to be associated with the development of cancer in approximately 200,000 patients worldwide each year. Therefore, work is underway on an EBV vaccine [3,4].

The only known reservoir of EBV are humans, and over 95% of the worldwide adult population have serological features of past infection [5,6]. Transmission occurs through saliva (kissing, coughing, sharing of food), genital secretions, blood transfusion, and transplantation [6]. The incubation period lasts from 30 to 50 days [6]. It is known that high contagiousness of EBV may occur up to 180 days after the onset of symptoms [7]. Young children and teenagers are most often affected.

Usually, the course of IM is self-limiting with an excellent prognosis. In approximately 80–90% of cases, the infection has mild or no symptoms, particularly among young children [6,8]. The older the child is, the greater the risk of full-blown IM with fever, tonsillitis, lymphadenopathy, fatigue, and hepatosplenomegaly [9]. The symptoms last for about two to four weeks.

According to the literature review (most of the data relate to adult patients), primary EBV infection is in 80–90% of cases associated with hepatic involvement, which is characterized by a mild to moderate acute elevation of liver aminotransferases as an effect of hepatocellular injury [8]. These abnormalities often persist unrecognized. The underlying pathogenesis and immune mechanisms remain unclear. No standard diagnostic criteria or management recommendations are available. The severity of the condition varies from asymptomatic, self-limited icteric hepatitis to acute liver injury (in rare cases). Patients diagnosed with hepatitis due to primary EBV infection may develop cholestatic features. Adult patients present with jaundice more often than children. Hemolysis or cholestasis are potential causes of jaundice in IM. The condition is usually benign and resolves spontaneously within a few weeks [9]. However, little information is available about children and adolescents with hepatitis due to primary EBV infection.

The aim of the study was to characterize pediatric patients diagnosed with hepatitis associated with primary EBV IM. Our study was conducted on a fairly large cohort of patients. Most studies to date concern much smaller groups. The assumption of this work was to create a general characteristic of the study population. We described the changes in the activity of liver aminotransferases during the infection, and we analyzed the results of abdominal ultrasonography evaluation.

2. Patients and Methods

2.1. Patients

We retrospectively analyzed the data collected on patients aged 0–18 years managed for IM due to primary EBV infection at the Department of Children's Infectious Diseases, Medical University of Warsaw, Regional Hospital of Infectious Diseases in Warsaw, between August 2017 and March 2023.

The onset of the disease was defined as the first day of fever, lymphadenopathy, sore throat, gastrointestinal symptoms, or jaundice. To confirm the primary EBV infection, we performed a serological test detecting immunoglobulin M antibody to EBV viral capsid antigen: anti-EBV VCA IgM (chemiluminescence immunoassay—CLIA test system, LIAISON EBV IgM, DiaSorin S.p.A., Saluggia, Italy) as a marker, which does not occur in chronic disease. The diagnosis of hepatitis related to primary EBV infection was made based on the increase of serum alanine aminotransferase (ALT) activity over age reference values [10].

We also performed more laboratory tests to assess liver function: serum aspartate aminotransferase and γ-glutamyl transferase (γ-GT) activity and bilirubin concentration with fractions. The laboratory investigation also included a full blood count with differential and C-reactive protein (CRP). We defined leukocytosis according to age and sex standards [11].

Abdominal ultrasonography was ordered in patients with a more severe course of the disease. Splenomegaly was diagnosed when the longitudinal dimension of the spleen exceeded the 97.5th percentile for age [12].

The following exclusion criteria were established:

(1) CMV co-infection (positive CMV IgM),
(2) pre-existing hepatitis or other underlying diseases with impaired liver function,
(3) positive tests result for other viruses (hepatitis A virus—HAV, hepatitis B virus—HBV, hepatitis C virus—HCV) causing similar symptoms,
(4) pre-existing medication, which could impair liver function.

2.2. Statistical Analysis

We analyzed collected medical records using STATISTICA 13.3 (StatSoft, Kraków, Poland). Data were presented as mean (Standard Deviation; SD) unless otherwise indicated. The chi-square (χ^2) test was used to verify the normality of the variables' distribution. Since none of the tested variables showed a normal distribution, the differences over time were compared through the non-parametric Kruskal-Wallis test for multiple comparisons. A two-tailed *p*-value of <0.05 was determined to be statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics

The data of 415 pediatric patients hospitalized due to IM syndrome within 79 months were retrospectively analyzed. In this group, 199 (47.9%) patients developed hepatitis with elevated ALT activity. Of these patients, 33 (16.6%) were excluded from the study because of coinfection with CMV (n = 27) and potentially hepatotoxic medication due to underlying chronic disease (n = 6) (Figure 1).

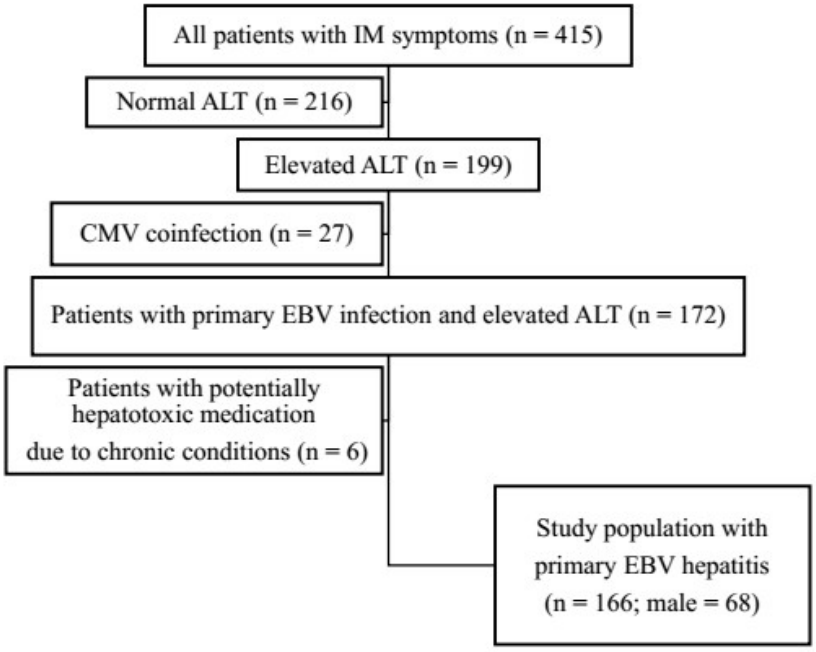

Figure 1. Flowchart showing the enrollment of patients. EBV, Epstein-Barr virus; ALT, alanine aminotransferase; CMV, cytomegalovirus.

One hundred sixty-six patients, aged 18 months to 18 years (mean, 11.9 ± 5.1 years), were enrolled in the study. Sixty-eight (40.9%) were male. There is an increased incidence of IM in two age groups: from 4 to 10 years (46 patients) and between 14 and 18 years of age (80 children). The age distribution is shown in Figure 2.

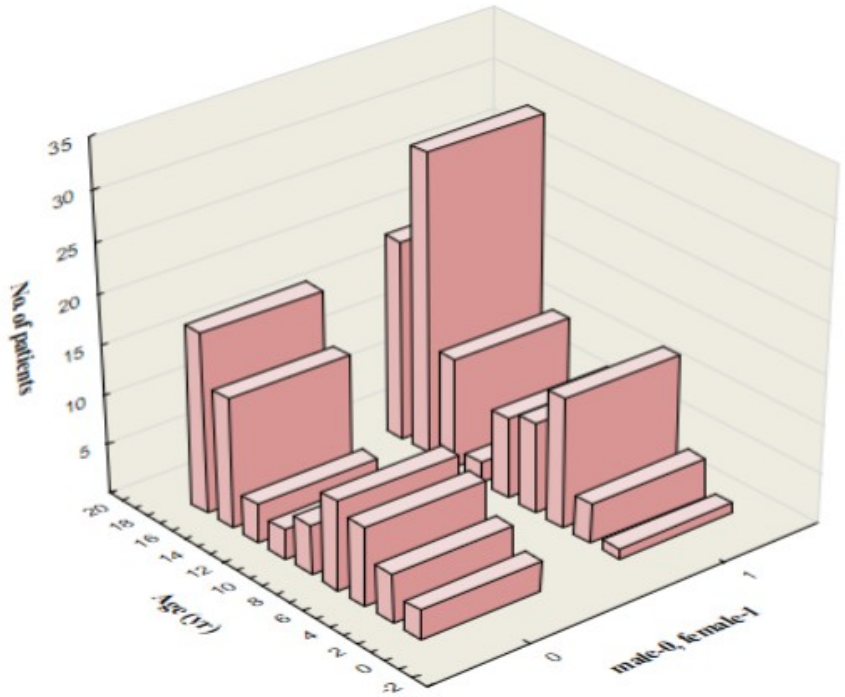

Figure 2. Age distribution in the study population (166 patients) by sex of the patients (male = 68). EBV, Epstein-Barr virus.

Forty-two (25.3%) patients suffered from other (chronic) diseases. Allergic conditions (bronchial asthma, allergic rhinitis, atopic dermatitis, and inhalant or food allergy) were the most common and concerned 23 (13.8%) patients. The second most common chronic disease was adenoid hyperplasia which occurred in five (3.0%) children.

3.2. Clinical Course and Complications

We analyzed the frequency of symptoms and signs in the study population. The average duration of symptoms was 14.4 ± 5.3 days and ranged from 4 to 30 days. The mean duration of hospitalization was 4.7 ± 2.2 days and ranged from 1 to 14 days.

The most common sign was lymphadenopathy, which was observed in 154 (92.8%) patients. Most often, the cervical lymph nodes were involved. Pharyngitis and tonsillitis with tonsillar exudates were the second most common clinical features and occurred in 150 (90.3%) patients. One hundred forty-three (86.1%) patients developed fever with an average duration of 8.0 ± 4.2 days (from 2 to 21 days). The classic triad of IM symptoms, including fever, lymphadenopathy, and pharyngitis or tonsillitis, was seen in 126 (75.9%) children.

Hepatomegaly and splenomegaly were noted in 134 (80.7%) and 111 (66.9%) patients, respectively. The concomitant enlargement of the liver and spleen presented 106 (63.8%) children. The degree of liver enlargement on palpation ranged from 0.5 to 10 cm (mean, 2.5 ± 1.3 cm) below the costal arch. Seventy-seven (46.4%) patients suffered from gastrointestinal symptoms such as abdominal pain and nausea or vomiting. A rash was seen in 55 (33.1%) children; among them, 28 (50.9%) developed an allergic-toxic generalized rash after previous amoxicillin administration. The frequency of signs and symptoms is presented in Table 1.

Table 1. Frequency of signs and symptoms in 166 patients with primary EBV hepatitis.

Signs and Symptoms	No. of Patients (%)
Lymphadenopathy	154 (92.8)
Pharyngitis with tonsillar exudate	150 (90.3)
Fever	143 (86.1)
Hepatomegaly	134 (80.7)
Splenomegaly	111 (66.9)
Rash	55 (33.1)
Abdominal pain	52 (31.3)
Eyelid swelling	49 (27.7)
Nausea or vomiting	45 (27.1)
Rash after amoxicillin administration	28 (16.9)
Jaundice/icteric sclera	19 (11.4)
Pruritus	19 (11.4)
Abdominal tenderness	18 (10.8)
Dark urine	8 (4.8)
Acholic stools	2 (1.2)

Concomitant infections were recognized in eighty-three patients (50.0%). The most common diagnosis was bacterial pharyngitis (based only on the clinical picture or confirmed by microbiological testing). Four patients were diagnosed with cervical lymphadenitis (2.4%), and three (1.8%) children developed sepsis. The incidence of the accompanying infections is shown in Table 2.

Table 2. The incidence of concomitant infections in 166 patients with primary EBV hepatitis.

Infection	No. of Patients (%)
Bacterial pharyngitis	51 (30.7)
Acute otitis media	8 (4.8)
Oral candidiasis	7 (4.2)
Scarlet fever	5 (3.0)
Pneumonia	4 (2.4)
Lymphadenitis	4 (2.4)
COVID-19	3 (1.8)
Sepsis	3 (1.8)
Urinary tract infection	3 (1.8)
Herpes labialis	2 (1.2)
Herpes zoster	1 (0.6)
Varicella	1 (0.6)
Influenza	1 (0.6)

Eighty patients (48.2%) developed complications not related to the liver, biliary tract, or gallbladder, which occurred before or during hospitalization. The obturation of the upper respiratory tract due to severe nasopharyngeal and palatal tonsils enlargement was the most common and was observed in 69 (41.5%) children. In four (2.4%) patients, the EBV infection was complicated with thrombocytopenia without purpura. Table 3 presents the incidence of particular complications.

Table 3. The incidence of particular complications other than the liver, biliary tract, or gallbladder pathology.

Complication	No. of Patients (%)
Obturation of the upper respiratory tract	69 (41.5)
Thrombocytopenia with or without purpura	4 (2.4)
Urticaria	3 (1.8)
Epistaxis	2 (1.2)
Syncope/loss of consciousness	2 (1.2)
Chest pain	2 (1.2)
Anemia	1 (0.6)
Iatrogenic inflammation and venous thrombosis	1 (0.6)
Vertigo	1 (0.6)

None of the study patients developed fulminant hepatitis, and no cases of EBV-hepatitis-related mortality were noted.

3.3. Laboratory and Imaging Test Results

The leucocyte count was evaluated in all patients (166) with values ranging from 4470 to 49,000 cells/μL (mean 14,621 ± 6888). Leukocytosis with lymphocytosis was observed in 129 patients (77.7%); thirty-three (19.9%) children had normal white blood cells count with relative lymphocytosis. The median lymphocyte percentage in white blood cell smear was 67.7 ± 13.6%, and atypical lymphocytes: 25.6 ± 14.5%. The lowest number of platelets was 9800, and the highest—was 455,000 (mean 209,823 ± 66,780).

We assessed inflammation markers: C-reactive protein (CRP)—in 161 patients and procalcitonin (PCT)—only in children with a more severe course of the disease. CRP ranged from normal values (<5 mg/dL) to 75 mg/dL. PCT serum concentration ranged from 0.05 to 37.0 ng/mL.

The activity of lactate dehydrogenase (LDH) in serum was assessed only in 20 patients with a mean value of 686.95 ± 232.25 U/L. Table 4 shows laboratory values on admission.

Table 4. Laboratory values on the admission of the 166 patients with EBV-hepatitis.

Laboratory Value	Mean (±SD)	No. of Patients
White blood cells count (cells/μL)	14,621 (6,8)	166
Neutrophils (%)	31.9 (13.2)	162
Lymphocytes (%)	67.7 (13.6)	162
Atypical lymphocytes (%)	25.6 (14.5)	162
Platelets (cells/μL)	209,823 (66,7)	166
CRP (mg/dL)	2.8 (16.4)	161
PCT (ng/mL)	1.92 (6.9)	28
LDH (U/L)	686.95 (232.2)	20

The activity of both enzymes ALT and AST were measured once or more in 164 (98.8%), twice or more in 83 (50.0%) and three or more times in eight (4.8%) children. Table 5 presents the number and percentages of patients with different values of hepatic laboratory parameters in the course of the disease.

Table 5. Number and percentages (%) of patients with different values of ALT, AST, γ-GT, and total bilirubin during EBV infection.

		Number of Patients (%)			
		ALT	**AST**	**γ-GT**	**Total Bilirubin**
1st week [a]	Normal	0 (0)	0 (0)	0 (0)	12 (52.2)
	<2×	12 (12.6)	24 (25.5)	1 (5.6)	4 (17.4)
	2–5×	39 (41.1)	42 (44.7)	5 (27.8)	4 (17.4)
	>5×	44 (46.3)	28 (29.8)	12 (66.6)	3 (13.0)
2nd week [b]	Normal	3 (2.6)	7 (6.5)	0 (0)	9 (50.0)
	<2×	19 (16.2)	30 (27.7)	2 (8.4)	2 (11.1)
	2–5×	53 (4.3)	38 (35.2)	5 (20.8)	5 (27.8)
	>5×	42 (35.9)	33 (30.6)	17 (70.8)	2 (11.1)
3rd week [c]	Normal	1 (1.8)	2 (4.2)	0 (0)	4 (66.6)
	<2×	7 (13.0)	15 (31.2)	0 (0)	2 (33.4)
	2–5×	24 (44.5)	19 (39.6)	3 (27.3)	0 (0)
	>5×	22 (40.7)	12 (25.0)	8 (72.7)	0 (0)
4th week [d]	Normal	2 (10.0)	3 (18.7)	0 (0)	2 (66.6)
	<2×	6 (30.0)	7 (43.8)	2 (33.4)	1 (33.4)
	2–5×	9 (45.0)	4 (25.0)	3 (50.0)	0 (0)
	>5×	3 (15.0)	2 (12.5)	1 (16.6)	0 (0)
>4 weeks [e]	Normal	1 (50.0)	0 (0)	0 (0)	0 (0)
	<2×	0 (0)	1 (33.4)	0 (0)	0 (0)
	2–5×	0 (0)	2 (66.6)	0 (0)	0 (0)
	>5×	1 (50.0)	0 (0)	0 (0)	0 (0)

[a] ALT was evaluated in 95 patients, AST was evaluated in 94 patients, γ-GT was evaluated in 18 patients, and total bilirubin was evaluated in 23 patients; [b] ALT was evaluated in 117 patients, AST was evaluated in 108 patients, γ-GT was evaluated in 24 patients, and total bilirubin was evaluated in 18 patients; [c] ALT was evaluated in 54 patients, AST was evaluated in 48 patients, γ-GT was evaluated in 11 patients, and total bilirubin was evaluated in 6 patients; [d] ALT was evaluated in 20 patients, AST was evaluated in 16 patients, γ-GT was evaluated in 6 patients, and total bilirubin was evaluated in 3 patients; [e] ALT was evaluated in 2 patients, AST was evaluated in 3 patients, γ-GT was evaluated in 0 patients, and total bilirubin was evaluated in 0 patients.

An average of 1.71 (±0.84) evaluation of AST activity was performed in 164 (98.8%) patients before or during hospitalization. The changes over time of mean AST activity showed statistical significance ($p = 0.0124$). Elevated AST levels were observed even in the first week of the illness and persisted until the fourth week showing two peaks: in the first (mean 186.34 ± 148.17 IU/L) and third (mean 169.00 ± 140.98 IU/L) week. A decreasing tendency was noted from the fourth week, with normalization in the fifth week from the onset of the disease.

ALT was measured on average 1.79 (±0.86) times for each child. ALT levels showed similar variability over time as AST, but there were no peaks of activity observed—ALT remains at a comparable elevated level for the first three weeks of the disease (mean 234.16 ± 164.77 IU/L).

γ-GT evaluation was performed from one to four times (mean 1.64 ± 0.72) in thirty-six patients. Mean γ-GT activity was above the upper normal limit in the first 3 weeks of the disease onset, reaching the highest values in the second week (mean 183.84 ± 97.48 IU/L).

Total serum bilirubin concentration was evaluated from one to three times (mean 1.26 ± 0.55) in thirty-eight patients; in twenty-three of them (60.5%), it exceeded the upper limit of the laboratory norm. Mean total bilirubin was elevated in the first two weeks of the disease (mean 2.0 ± 2.00 mg/dL), and normalization was observed from the third week.

In 18 (47.4%) patients, conjugated bilirubin was at least 20% of total bilirubin, all of them had elevated activity of γ-GT and prothrombin ratio in normal ranges from 72 to 123% (mean 88.9 ± 9.6%). In this group (10.8% of the study population), transient cholestatic liver disease was the predominant type of hepatic involvement. All of them presented with jaundice, pruritus, and abdominal tenderness; eight (44.4%) children reported darkening of the urine and two (11.1%)—acholic stools. The mean age in the group of patients with cholestatic features was 13 ± 5.23 years, and 12 (66.6%) children were older than 15 years (Figure 3).

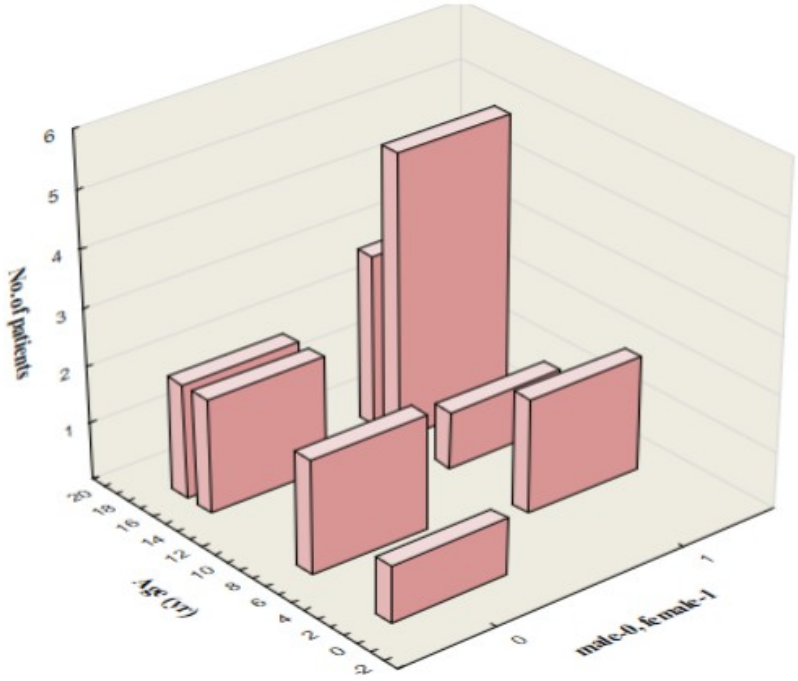

Figure 3. Age distribution by sex of 18 patients with cholestatic liver disease in primary EBV hepatitis (male = 7). EBV, Epstein-Barr virus.

ALT activity and total serum bilirubin concentration exceeding five times the upper limit of the laboratory norm were noted mainly in the first week of the disease in 46.3% and 13% of patients, respectively (ALT level and total serum bilirubin concentration were evaluated in the first week of the disease in 95 and 23 patients, respectively). AST and γ-GT activity more than five times the upper limit of the laboratory norm occurred predominantly in the second week of illness and occurred in 30.6% and 70.8% of children, respectively (the above parameters were measured in the second week of the disease in 108 and 24 patients, respectively). Changes in ALT, AST, γ-GT, and total bilirubin levels during the disease are shown in Figure 4.

The abdominal ultrasound was performed in 116 (69.8%) patients, and abnormalities were observed in 111 (66.8%) cases. Splenomegaly was the most common cause of abnormal results and occurred in 99 (85.3%) children. Hepatomegaly was shown in 72 (43.4%) children, and enlargement of the lymph nodes of the hepatic hilum—in 64 (38.5%) cases. In 97 (83.6%) out of 116 patients who underwent abdominal ultrasonography, an enlarged liver was found on palpation. This finding was confirmed by abdominal ultrasound in 64 (65.9%) children. Eight patients (6.9%) were diagnosed with hepatomegaly by ultrasonography, which was not detected by palpation. Abnormalities related to the gallbladder and biliary

tract were shown in 19 (11.4%) patients, and most often occurred the gallbladder wall thickening—in 17 (10.2%) cases. Gallbladder polyps and fluid collection in the hepatic hilum were observed in two (1.2%) and three (1.8%) patients, respectively.

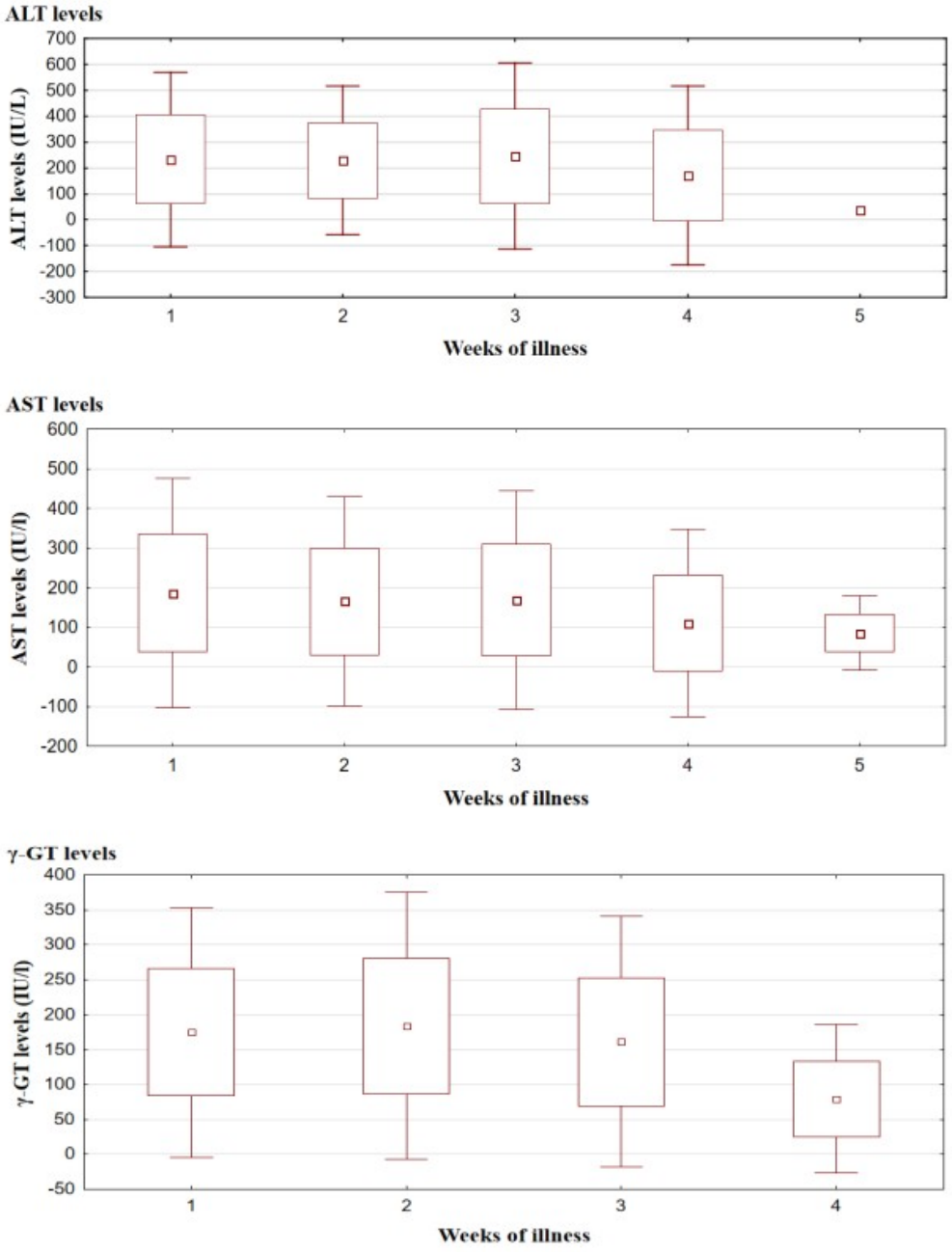

Figure 4. *Cont.*

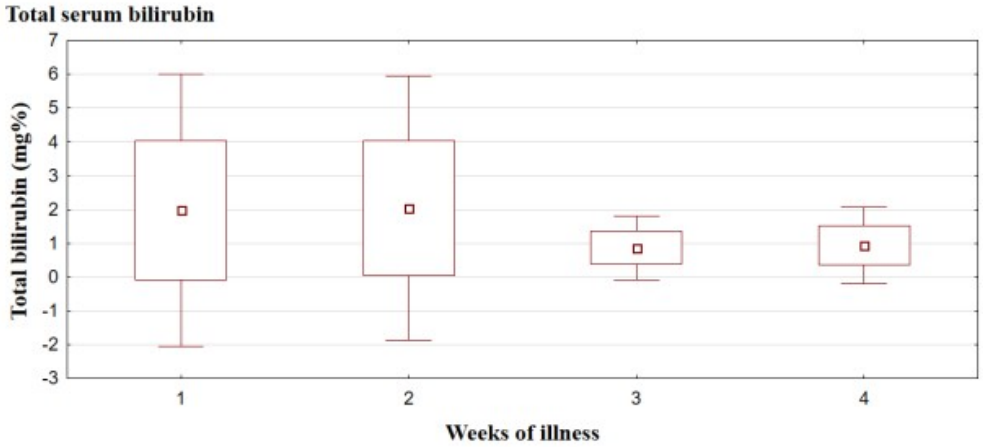

Figure 4. Mean activity of ALT, AST, γ-GT, and total serum bilirubin concentration (+/− standard deviation) during EBV infection. Kruskal-Wallis test was used for changes over time: $p = 0.0124$ for AST. No significant changes over time for ALT ($p = 0.0581$), γ-GT ($p = 0.0988$), and total serum bilirubin ($p = 0.8562$). ALT—alanine-aminotransferase; AST—aspartate-aminotransferase; γ-GT—γ-glutamyl transferase.

3.4. Treatment

All patients required supportive care due to high fever, dehydration, upper respiratory tract obturation, or severe allergic-toxic rash after amoxicillin.

Ninety-one (54.8%) patients received an empirical antimicrobial therapy before the hospitalization (and before the establishment of the diagnosis), most often due to pharyngitis. Among them, 23 (25.6%) were treated with several antibiotics: 24 (26.4%) received two antibiotics, and 4 (4.4%) received three. Forty-three (47.2%) children were prescribed amoxicillin, and 28 (65.1%) of them developed an allergic-toxic rash 7–10 days after the first dose of the medication. The number (and percentage) of patients treated with particular antibiotics are presented in Table 6.

Table 6. The number (and percentage from ninety-one cases) of patients treated with particular antibiotics before hospitalization.

Antibiotic	No. of Patients (%)
Cefuroxime axetil	31 (34.1)
Amoxicillin with clavulanic acid	29 (31.8)
Phenoxymethylpenicillin	16 (17.6)
Azithromycin	15 (16.5)
Amoxicillin	14 (15.4)
Cefadroxil	6 (6.6)
Clarithromycin	5 (5.5)
Trimethoprim/Sulfamethoxazole	1 (1.1)
Cefazoline	1 (1.1)
No data on a specific preparation	2 (2.2)

During the hospitalization, 85 (51.2%) children required antibiotic therapy. The most common indication for the antimicrobial regimen was concomitant bacterial pharyngitis.

Five patients (5.8%) received two antibiotics. Table 7 shows the number of patients treated with particular antibiotics during the hospitalization.

Table 7. The number (and percentage from eighty-five cases) of patients treated with particular antibiotics during the hospitalization.

Antibiotic	No. of Patients (%)
Cefuroxime or Cefuroxime axetil	66 (77.6)
Clindamycin	8 (9.4)
Clarithromycin	6 (7.0)
Penicillin G or Phenoxymethylpenicillin	4 (4.7)
Ceftriaxone	4 (4.7)
Azithromycin	2 (2.3)
Metronidazole	2 (2.3)

In 27 (29.7%) of the ninety-one patients previously treated with antibiotics, the therapy was continued using the same medication, and in 25 (27.5%) patients, the therapy was changed. The antibiotic therapy was discontinued in 39 (42.8%) children because of its ineffectiveness or occurrence of side effects (for example, allergic-toxic rash after amoxicillin). Thirty-three (19.8%) patients required initiation of antibiotic therapy.

4. Discussion

In our retrospective study, we characterized a group of 166 pediatric patients diagnosed with EBV-hepatitis. The age distribution in the examined population indicates the dominance of two age groups: 4–8 years and 14–18 years. In the first group of 41 patients (24.7%), there is a slight female predominance (53.6%). This trend was more pronounced in the second group of 90 patients aged 14–18, where girls constitute 62.2%.

The pathogenesis of EBV-hepatitis remains unclear. Infection of hepatocytes with primary hepatotropic viruses, such as hepatitis B or C, has no direct cytopathic effect, and symptoms of liver injury result from an immune response to viral antigens presented by infected hepatocytes [13,14]. Hepatocytes, biliary and sinusoidal epithelium are not the target cells for EBV [15]. The possible pathogenetic mechanism of parenchymal injury of the liver tissue and cholestasis in EBV infection involves the effect of the virus on the systemic and intrahepatic synthesis of pro-inflammatory cytokines that affect the sinusoidal and tubular bile transport systems. EBV-infected CD8+ T cells in the liver are known to secrete inflammatory mediators such as tumor necrosis factor α, interferon-γ, and Fas ligand. Another explanation could be an inhibition of antioxidant mechanisms through the production of autoantibodies [15,16].

Available literature data in children indicate that 80–90% of patients with primary EBV infection have clinical and/or laboratory evidence of hepatitis [8,17,18]. Our study demonstrated that liver involvement during acute EBV infection develops in approximately 50% of hospitalized children, which is inconsistent with previous reports. In our study cohort, hepatitis was mild and self-limited, and cholestatic features were present in 10.8% of patients with hepatitis. It is known that cholestatic hepatitis in primary EBV infection occurs more commonly with increasing age and has been reported mainly in adults [19]. In our study, 66.6% of patients with clinical and laboratory features of cholestasis were older than 15 years, which is consistent with previous reports.

Forty (24.1%) patients did not present the classic triad of IM symptoms (fever, lymphadenopathy, and pharyngitis or tonsillitis).

The abnormalities of ALT and AST serum activity in our study were observed from the first to the third week of the disease and started returning to normal in the fourth week. Our findings are consistent with the existing literature [8,20]. Values of the above parameters exceeding five times the upper limit of the laboratory norm were most often

observed in the first week of the disease for ALT (46.3% of patients) and in the second week for AST (30.6%). These results are, in part, inconsistent with studies in the adult population, which state that serum aminotransferase levels are usually elevated by less than five times the upper normal limit [17,18,21].

Our study demonstrated two peaks in AST levels: in the first and third week of illness. A similar pattern of changes in AST activity over time was described in 41 adult patients by Kofteridis et al.

Abdominal ultrasonography in EBV infection may reveal splenomegaly, hepatomegaly, porta hepatis adenopathy, periportal edema, and fluid collection or gallbladder wall thickening (GBWT). A gallbladder wall thickness greater than 3 mm is considered abnormal [22]. The last of the listed abnormalities is recognized as a sign of the severity of hepatitis. A possible explanation could be an immune reaction (as seen in hepatitis) or swelling of the gallbladder wall as a result of lymphatic obstruction due to the enlargement of porta hepatis lymph nodes [23]. Dehydration as a result of pharyngitis and poor appetite can also increase bile viscosity.

GBWT greater than 3 mm in the absence of cholelithiasis in patients with abdominal pain localized in the right upper quadrant is defined as acute acalculous cholecystitis (AAC). Such gallbladder inflammation accounts for approximately 30–50% of cholecystitis in the pediatric population [24]. Abdominal ultrasonography in AAC may reveal biliary sludge, gallbladder distention (hydrops), or pericholecystic fluid as well. AAC as a complication of viral infection in children is rare but was reported in primary CMV infection, EBV infection, and human herpes virus type 6 infection [25–29].

In our study, 18 patients presented ultrasonographic signs of AAC, but only three of them reported abdominal pain localized in the right upper quadrant. These three patients were females older than 16 years. It is consistent with previous reports, which describe female predominance in AAC in both adult and pediatric populations with EBV infection [27]. AAC, as a complication of EBV infection, is usually associated with a favorable prognosis [30].

Immunocompetent patients with symptomatic EBV infection usually spontaneously recover with supportive care (antipyretics, analgesics, hydration, and rest). In our study, children with complications in the form of upper respiratory tract obturation received systemic corticosteroids. Antibiotics were administered in cases of concomitant signs and symptoms of bacterial infection, especially in the presence of significantly elevated inflammatory markers such as CRP and PCT or microbiological confirmation. All our patients achieved full recovery without any chronic complications.

In conclusion, although our study has some limitations, namely its retrospective nature and unavailability of all laboratory data, it demonstrates that primary EBV hepatitis is mostly a mild and transient condition. No case of acute liver failure was reported in this study group. In the available literature, isolated cases of fatal liver failure—even in immunocompetent patients—have been reported [31]. Therefore, it is important to determine the activity of liver enzymes and monitor them in patients with a more severe course of IM. Delayed presentation of patients to the physician may have a negative impact on our results. Not all children had performed additional tests at the beginning of the disease. We analyzed the course of hepatitis only in children requiring hospitalization due to primary EBV infection.

Author Contributions: Conceptualization, M.R. and M.P.-Ś.; Data curation, M.R.; Formal analysis, M.R. and M.P.-Ś.; Investigation, M.R.; Methodology, M.R.; Project administration, M.R.; Resources, M.R. and M.P.-Ś.; Software, M.R.; Supervision, M.P.-Ś.; Visualization, M.R. and M.P.-Ś.; Writing—original draft, M.R.; Writing—review & editing, M.R. and M.P.-Ś. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study because the study was retrospective, non-interventional and based on data collected in the Department's

database. Therefore, it does not require approval of the Ethics Committee. Due to the retrospective nature of the presented study, written consent by participants was not necessary. The patients' data was protected according to the European Union General Data Protection Regulation.

Informed Consent Statement: Patient consent was waived due to the retrospective nature of the presented study. The patients' data was protected according to the European Union General Data Protection Regulation.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available, because the study based on data collected in the Department's database.

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

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Publikacja nr 2.

Rutkowska M, Pokorska-Śpiewak M.

The influence of steroid therapy of complications of infectious mononucleosis on the course of Epstein-Barr virus hepatitis. Clin Exp HEPATOL. 2023; 9 (4): 375–385

Clinical and Experimental HEPATOLOGY

Clin Exp HEPATOL 2023; 9, 4: 375–385
DOI: https://doi.org/10.5114/ceh.2023.133169
Received: 16.09.2023, **Accepted:** 17.10.2023, **Published:** 24.11.2023

Original paper

The influence of steroid therapy of complications of infectious mononucleosis on the course of Epstein-Barr virus hepatitis

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Abstract

Aim of the study: The purpose of the study was to characterize the differences in the course of Epstein-Barr virus (EBV) primary infection-induced hepatitis between patients treated with steroids due to complications of infectious mononucleosis (IM) and those not receiving such therapy.

Material and methods: We analyzed the changes in the activity of liver enzymes and differences in abdominal ultrasound results. The study was based on reviewing the medical records of children hospitalized for primary EBV infection at the Department of Children's Infectious Diseases, Medical University of Warsaw, Regional Hospital of Infectious Diseases in Warsaw, between August 2017 and March 2023. The study population was divided into two groups: patients treated with steroids (Group 1) and children not receiving steroids (Group 2).

Results: Significant differences were obtained for alanine aminotransferase activity only in the first week of IM (205.34 ±115.40 vs. 288.82 ±170.16 IU/l for Group 1 and 2, respectively; $p = 0.024$), and for aspartate aminotransferase in the first (170.63 ±159.47 vs. 218.85 ±128.22 IU/l for Group 1 and 2, respectively; $p = 0.009$) and the third week (151.09 ±138.57 vs. 235.50 ±170.27 IU/l for Group 1 and 2, respectively; $p = 0.016$). The analysis of the results of laboratory tests for the diagnosis of cholestasis (γ-glutamyl transferase and total serum bilirubin concentrations with fractions) did not show significant differences between the groups.

Conclusions: Our results indicated that the two cohorts of patients may differ in the course of hepatitis associated with primary EBV infection, especially at the beginning of the disease, when the laboratory features of hepatitis were less pronounced in children treated with steroids.

Key words: steroids, viral hepatitis, alanine aminotransferase, aspartate aminotransferase, Epstein-Barr virus.

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Introduction

Infectious mononucleosis (IM) is considered a benign lymphoproliferative disorder caused by the Epstein-Barr virus (EBV, human gamma-herpesvirus 4 – HHV-4). EBV is widespread in all human societies and cultures (humans are the only known reservoir of this pathogen), and approximately 95% of the world's population has detectable IgG antibodies against EBV [1-3]. Infection occurs through close contact with an EBV-shedder: by sharing food or utensils, kissing, coughing, and sneezing (contact with saliva) or sexual intercourse, as well as after blood transfusions or organ transplantations [2]. The risk of infection is estimated at 10% [4]. About 10-20% of infected people develop full-blown IM preceded by a 30-50-day incubation period [2]. In the remaining infected persons (usually young children), the condition is mild or asymptomatic [2, 5]. Primary infection results in a life-long latency of EBV in memory B cells. The virus can periodically (during reactivation) spread in saliva throughout the life of an

infected person [3, 6, 7]. Contagiousness lasts up to 6 to 18 months from the onset of the disease.

The classic triad of IM symptoms includes fever, pharyngitis, and lymphadenopathy. Patients may present with other signs and symptoms, such as fatigue, nausea, vomiting, anorexia, rash, hepatosplenomegaly, and abdominal pain. The condition is usually self-limiting and resolves within 2 to 3 weeks. Eighty to ninety percent of patients with primary EBV infection develop hepatitis with mild to moderate elevation of liver aminotransferases [5]. Liver enzyme values usually return to normal within a few weeks [8]. Symptoms of cholestasis may appear. EBV infection is a potential cause of acalculous cholecystitis as well [9]. Isolated cases of fatal liver failure in immunocompetent patients have been reported in the available literature [10].

The pathomechanism of parenchymal liver injury and cholestasis in primary EBV infection remains unclear. An important role may be played by EBV-infected $CD8^+$ T cells in the liver, which secrete inflammatory mediators such as tumor necrosis factor α, interferon γ, and Fas ligand with a potential impact on the sinusoidal and tubular bile transport systems [11, 12]. EBV infection is not localized in hepatocytes and biliary cells.

Treatment of IM is symptomatic and includes adequate hydration and management of fever and pain with nonsteroidal anti-inflammatory drugs or acetaminophen. Patients who develop dehydration or complications such as difficulty breathing due to swelling of the pharyngeal lymphatic tissue or more severe hepatitis may require hospitalization.

To date, no target antiviral therapy has been developed. Acyclovir is not recommended – a meta-analysis of five randomized controlled trials involving 339 patients showed no significant clinical benefit [13].

Steroids have been ordered for their anti-inflammatory effect since the 1950s, but there are no clear guidelines for their use in IM. The above action of glucocorticoids results from the direct and indirect modification of the expression of genes encoding pro-inflammatory factors. According to a literature review, no clinical benefit from the use of steroids in IM was found in 8/10 trials. Two studies have shown the benefit of steroid therapy over placebo in reducing sore throat at 12 hours, but this effect was not maintained [4]. Literature data indicate that corticosteroids may be given especially to patients who develop complications such as significant pharyngeal edema that threatens respiratory compromise, or autoimmune disorders: anemia, and thrombocytopenia [14]. On the other hand, the value of such treatment is controversial and steroids may delay clearance of the viral load [3].

Our study aimed to determine the differences in the course of hepatitis associated with primary EBV infection between the patients treated with steroids and those not receiving such therapy.

Material and methods

Patients

The study was based on a retrospective analysis of the medical records collected among patients aged 0-18 years hospitalized for IM due to primary EBV infection at the Department of Children's Infectious Diseases, Medical University of Warsaw, Regional Hospital of Infectious Diseases in Warsaw, between August 2017 and March 2023.

The onset of IM was considered to be the appearance of the first signs and symptoms such as swollen cervical lymph nodes, sore throat, fever, gastrointestinal complaints, or jaundice. The diagnosis was confirmed by a positive result of a serological test detecting immunoglobulin M antibody to EBV viral capsid antigen – anti-EBV VCA IgM (chemiluminescence immunoassay – CLIA test system, LIAISON EBV IgM, DiaSorin S.p.A., Saluggia, Italy) – as a marker of acute infection.

Hepatitis associated with primary EBV infection was diagnosed based on the elevation of serum alanine aminotransferase (ALT) activity over age reference values [15]. In addition, the following laboratory tests were performed: serum aspartate aminotransferase (AST) and γ-glutamyl transferase (γ-GT) activity, and total serum bilirubin concentration with fractions. The main indication for determining the activity of γ-GT and total serum bilirubin concentration with fractions was symptoms of cholestasis: jaundice, pruritus, acholic stools, and dark urine. We also performed full blood count with differential and C reactive protein (CRP). Leucocytosis was defined according to age and sex standards [16].

A more severe course of the disease, especially with symptoms of cholestasis or markedly enlarged spleen and/or liver on palpation, was an indication for abdominal ultrasound. Splenomegaly was defined as the longitudinal dimension of the spleen exceeding the 97.5^{th} percentile for age [17].

We divided the study population – children and adolescents diagnosed with primary EBV hepatitis – into two groups: the first cohort included patients who received steroids, and the second consisted of patients not treated with steroids.

The most common indication for steroid therapy was significant airway obturation with sleep apnea or allergic-toxic rash after amoxicillin.

We established the following exclusion criteria: 1. Coexisting cytomegalovirus (CMV) infection (positive CMV IgM), 2. Pre-existing liver diseases of any etiology, 3. Diagnosis of other viral liver infections: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), 4. Prior pharmacotherapy with potential hepatotoxicity.

Statistical analysis

Statistical analysis of collected medical records was performed using STATISTICA 13.3 (StatSoft, Kraków, Poland). Data were presented as mean (standard deviation; SD) unless otherwise indicated. To compare clinical features between study groups, the χ^2 test (for categorical variables) was performed; in the case of an insufficient number of observations in the subgroups, Fisher's exact test was used. We analyzed the differences in laboratory findings, the duration of signs and symptoms, and the length of hospital stay (continuous variables) between the study groups using the Mann-Whitney *U* test. A *p*-value of < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

The medical records of 415 children and adolescents hospitalized due to IM associated with EBV infection within 79 months were retrospectively analyzed. In this cohort, 199 (47.9%) patients were diagnosed with hepatitis with increased ALT activity. Of this group, 33 (16.6%) were excluded from the study because of positive serological test results (IgM antibodies) for CMV ($n = 27$), and potentially hepatotoxic pharmacotherapy due to pre-existing chronic disease ($n = 6$). For the study, 166 patients, aged 18 months to 18 years (mean, 11.9 ±5.1 years), were recruited. In the first group of 104 patients (62.6%) who were treated with steroids the male-to-female ratio was 1 : 1.47. In the second cohort of 62 (37.3%) children who did not receive steroids the male-to-female ratio was 1 : 1.38.

Our patients received steroids intravenously, temporarily, mainly in the case of symptoms of upper respiratory tract obturation. Forty-eight children (46.1%) received dexamethasone, 47 (45.2%) hydrocortisone, and prednisolone were administered much less often – only in 8 patients (7.7%). Children up to 5 years of age were treated with hydrocortisone at a dose of 8-10 mg/kg/day, and older children at a dose of 4-8 mg/kg/day, in 3 divided daily doses. The dose of dexamethasone was 0.15-0.6 mg/kg/day in 4 divided daily doses. Prednisolone was administered at a dose of 1-4 mg/kg/day, in 3 divided daily doses. During hospitalization, patients received an average of 4.06 ±2.81 doses of steroids (from 1 to 14 doses). Most frequently, patients were administered steroids in the first and second weeks of illness.

The flowchart (Fig. 1) shows the enrolment of patients in the study.

The age distribution in the two groups of the study population is shown in Figure 2.

Clinical course and complications

Analyzing the clinical course of IM in Groups 1 and 2, we obtained significant differences regarding the length of hospitalization (5.0 ±2.1 vs. 4.3 ±2.3 days; $p = 0.015$), the occurrence of the classic triad of IM symptoms (which was observed in 84.6% of children treated with steroids and in 72.6% of patients without steroid therapy; $p = 0.000$), fever – the most common clinical feature (90.4% of patients in Group 1 vs. 79.0% in Group 2; $p = 0.040$), and its duration (patients received steroids presented with fever for 7.5 ±4.7 vs. 5.9 ±4.8 days in children without steroid therapy; $p = 0.049$). Significant differences were observed in the occurrence of hepatomegaly (85.5% of children in Group 1 vs. 72.6% in Group 2; $p = 0.040$), and splenomegaly (73.1% vs. 56.4%; $p = 0.027$) as well. As regards age, the male-to-female ratio, the duration of signs and

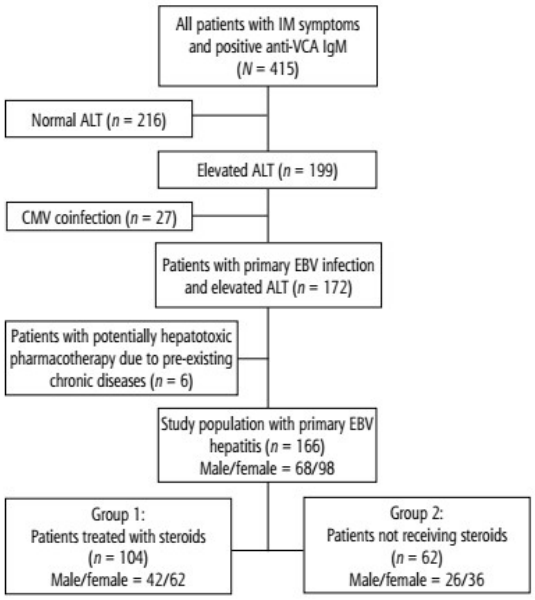

Fig. 1. Flowchart showing recruitment of patients to the study. IM – infectious mononucleosis, ALT – alanine aminotransferase, CMV – cytomegalovirus, EBV – Epstein-Barr virus

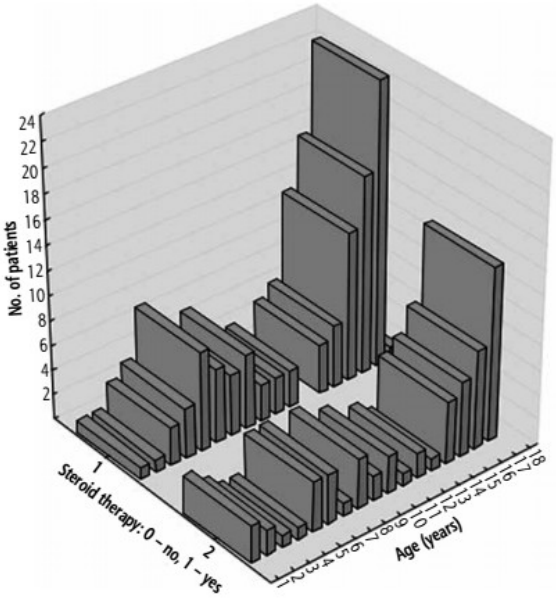

Fig. 2. Age distribution in Group 1 (n = 104) and Group 2 (n = 62) of patients with primary Epstein-Barr virus (EBV) hepatitis

Table 1. Differences in clinical features of infectious mononucleosis (IM) between study groups

Variable	Group 1 (n = 104)	Group 2 (n = 62)	P-value
	Mean ±SD		
Age (years)	12.1 ±4.9	11.7 ±5.2	0.766
Sex (male/female)	42/62	26/36	0.844
Duration of signs and symptoms (days)	14.3 ±4.8	14.6 ±6.2	0.807
Days of hospitalization	5.0 ±2.1	4.3 ±2.3	0.015
Fever duration (days)	7.5 ±4.7	5.9 ±4.8	0.049
	No. of patients (%)		
Fever	94 (90.4)	49 (79.0)	0.040
Classic triad symptoms of IM	88 (84.6)	38 (61.3)	0.000
Hepatomegaly on palpation	89 (85.5)	45 (72.6)	0.040
Splenomegaly on palpation	76 (73.1)	35 (56.4)	0.027
Abdominal pain	30 (28.8)	22 (35.5)	0.372
Abdominal tenderness	10 (9.6)	8 (7.7)	0.509
Jaundice	10 (9.6)	9 (8.6)	0.337
Pruritus	11 (10.6)	8 (7.7)	0.664
Dark urine	5 (4.8)	3 (4.8)	0.992
Acholic stools	1 (0.9)	1 (1.6)	0.709

symptoms of IM, the incidence of abdominal pain and abdominal tenderness on palpation, and symptoms of cholestasis (jaundice, pruritus, dark urine, and acholic stools), they showed no statistically significant differences between the groups of patients. The differences in clinical features between the study groups are shown in Table 1.

Sixty-five patients (62.5%) treated with steroids and 15 (24.2%) children who had not received steroids developed complications of IM (p = 0.000). Obturation of the upper respiratory tract as the main reason for steroid administration was obviously more frequently observed among patients in the first group – 58 (55.7%) – than in the second group – 11 (17.7%); p = 0.000. Other common complications occurred without a statistically significant difference: allergic-toxic rash after amoxicillin administration was reported in 21 (20.2%) children in Group 1, and 7 (11.3%) in Group 2; thrombocytopenia with or without purpura was observed in 4 (3.8%) patients only in patients treated with steroids.

Other complications were observed rarely, and are presented in Table 2.

Laboratory and imaging test results

The mean number of white blood cells (WBC) assessed in the peripheral blood count (performed in all patients) in both groups of patients did not differ and was 14.75 ±5.76 cells/μl in the first group vs 14.39 ±8.49 cells/μl in the second group. Leukocytosis was found in 85 (81.7%) patients treated with steroids and 44 (70.9%) children not receiving steroids (p = 0.107). A white blood cell smear was performed in 162 (97.6%) patients and statistically significant differences were obtained in the percentage of neutrophils (33.5 ±12.2% in Group 1 vs. 19.4 ±14.5% in Group 2; p = 0.020). CRP concentration – assessed in 161 (96.9%) patients – also

Table 2. Incidence of particular complications (other than the liver, biliary tract, or gallbladder pathology) in Groups 1 and 2

Complication	Group 1 (n = 104) No. of patients (%)	Group 2 (n = 62) No. of patients (%)
Urticaria	2 (1.9)	1 (1.6)
Epistaxis	2 (1.9)	0
Syncope/loss of consciousness	1 (0.9)	1 (1.6)
Chest pain	0	2 (3.2)
Anemia	0	1 (1.6)
Iatrogenic inflammation and venous thrombosis	1 (0.9)	0
Vertigo	0	1 (1.6)

Table 3. Comparison of laboratory data between study groups

Variable	Group 1 (n = 104) Mean ±SD	Group 2 (n = 62) Mean ±SD	P-value
WBC (cells/µl)	14.75 ±5.76	14.39 ±8.49	0.101
Neutrophils (%)[a]	33.5 ±12.2	19.4 ±14.5	0.020
Lymphocytes (%)[a]	66.1 ±12.8	70.7 ±14.4	0.013
Atypical lymphocytes (%)[a]	25.5 ±14.3	25.8 ±14.9	0.801
Platelets (cells/µl)[b]	208.70 ±63.72	211.73 ±72.19	1.000
CRP (mg/dl)[c]	25.51 ±17.92	15.46 ±11.11	0.000
PCT (ng/ml)[d]	2.74 ±8.86	0.06 ±0.55	0.706
LDH (U/l)[e]	613.5 ±185.66	735.92 ±254.35	0.231

[a] White blood cell smear showing the percentage of lymphocytes, neutrophils, and atypical lymphocytes was assessed in 162 patients; [b] Platelets were evaluated in 165 patients; [c] CRP – C reactive protein was evaluated in 161 patients; [d] PCT – Procalcitonin was assessed in 28 patients; [e] LDH – Lactate dehydrogenase was evaluated in 20 patients.

differed statistically significantly: 25.51 ±17.92 mg/dl vs. 15.46 ±11.11 mg/dl, respectively in the first and the second group; p = 0.000. There were no significant differences in the percentage of atypical lymphocytes, platelet count, and serum concentration of procalcitonin (PCT) and lactate dehydrogenase (LDH) in the study groups. Table 3 shows the comparison of laboratory data between study groups.

The analysis of ALT activity in both study groups in the successive weeks of the IM showed that in the first, second, and third weeks, ALT levels were higher in Group 2, and the highest values were observed in the first and third weeks of the disease. In the first group, the ALT level was the highest in the first week of IM, and a downward trend was observed in the following weeks, without a peak in the third week as in Group 2. Figure 3 shows the mean activity of ALT in successive weeks of EBV hepatitis in both groups of patients.

The medians of serum ALT activity in the study groups differed statistically significantly only in the first week of the disease, and their mean values were 205.34 ±115.40 IU/l (ranging from 39.00 to 516.00) vs. 288.82 ±170.16 IU/l (ranging from 50.00 to 725.00) for Groups 1 and 2, respectively; p = 0.024. ALT levels in the first week of IM were evaluated in 95 (57.2%) patients: 61 (58.6%) from the first group and 34 (54.8%) from the second group. The comparison of the median activity of ALT in both groups in the first week of the disease is shown in Figure 4.

The median ALT serum concentrations in the compared groups of patients in the successive weeks (from 2nd to 4th) of the disease did not differ statistically significantly. Mean ALT values assessed from the first to fourth week of IM in both study groups are shown in Table 4.

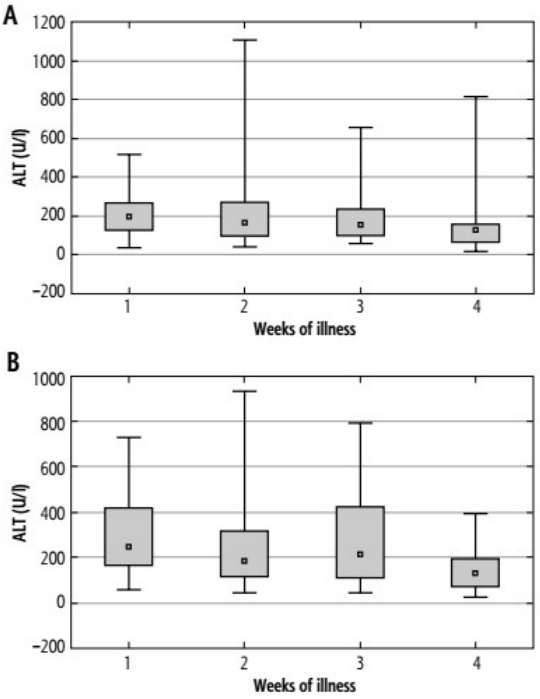

Fig. 3. Mean activity of alanine-aminotransferase (ALT) (± standard deviation) in successive weeks of Epstein-Barr virus (EBV) hepatitis in patients treated with steroids (Group 1), p = 0.129 (**A**), and in patients not treated with steroids (Group 2), p = 0.277 (**B**). The Kruskal-Wallis test was used for changes over time

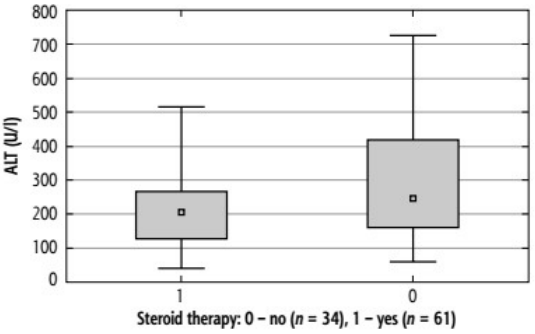

Fig. 4. Comparison of median activity of alanine aminotransferase (ALT) in the 1st week of infectious mononucleosis (IM) in Groups 1 and 2; p = 0.024 in the Mann-Whitney U test (n = 95)

Aspartate aminotransferase activity in the first three weeks of the disease was higher among patients not treated with steroids than in the patients undergoing this therapy. In both groups, slightly higher AST levels can be observed in the first and third weeks of IM. However, these differences are statistically significant only in children not receiving steroids (p = 0.024).

Table 4. Values of alanine aminotransferase (ALT) activity in Groups 1 and 2 in successive weeks of the disease

Variable	ALT (IU/l) in the 1st week		ALT (IU/l) in the 2nd week		ALT (IU/l) in the 3rd week		ALT (IU/l) in the 4th week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	61	34	72	45	30	24	11	9
Mean ±SD	205.34 ±115.40	288.82 ±170.16	207.90 ±162.43	249.37 ±187.94	210.56 ±152.73	291.75 ±212.65	178.36 ±217.67	167.44 ±132.29
Range	39-516	58-725	42-1105	43-932	59-660	42-791	24-814	26-390
p-value	0.024		0.284		0.321		0.790	

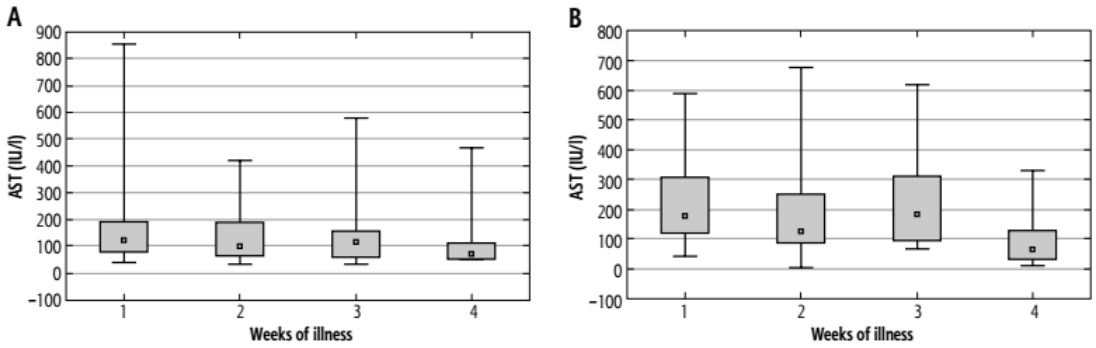

Fig. 5. Mean activity of aspartate-aminotransferase (AST) (± standard deviation) in successive weeks of Epstein-Barr virus (EBV) hepatitis in patients treated with steroids (Group 1), $p = 0.256$ (**A**), and in patients not treated with steroids (Group 2), $p = 0.024$ (**B**). The Kruskal-Wallis test was used for changes over time

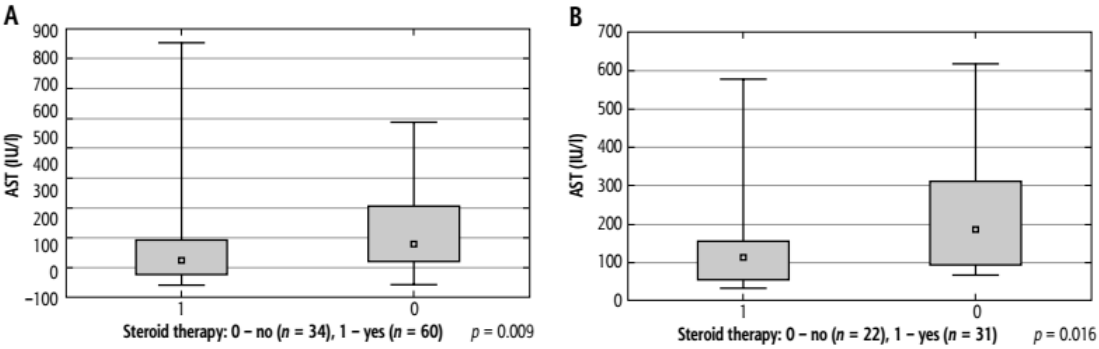

Fig. 6. Comparison of median activity of aspartate aminotransferase (AST) in Group 1 ($p = 0.009$) and Group 2 ($p = 0.016$) in the **A**) 1st ($n = 94$) and **B**) 3rd weeks ($n = 53$) of infectious mononucleosis (IM) in the Mann-Whitney *U* test

Figure 5 shows the mean activity of AST in successive weeks of EBV hepatitis in both groups of patients.

The median AST levels in the study groups differed statistically significantly in the first and third weeks of IM. The mean values of AST activity in the first week were: 170.63 ±159.47 IU/l (ranging from 40.00 to 852.00) vs. 218.85 ±128.22 IU/l (ranging from 42.00 to 589.00) for Groups 1 and 2, respectively; $p = 0.009$. The mean AST levels in the third week were: 151.09 ±138.57 IU/l (ranging from 31.00 to 578.00) vs. 235.50 ±170.27 IU/l (ranging from 67.00 to 617.00) for Groups 1 and 2, respectively; $p = 0.016$. AST activity in the first and third weeks of the disease was evaluated in 94 (56.6%) patients and 53 (31.9%) children, respectively. The comparisons of the median activity of AST in both groups in the first and third weeks of the disease are shown in Figure 6.

The median AST serum activity in the compared groups of children in the second and fourth weeks of the disease did not differ statistically significantly. Mean AST values evaluated from the first to fourth week of IM are presented in Table 5.

Table 5. Values of aspartate aminotransferase (AST) activity in Groups 1 and 2 in successive weeks of the disease

Variable	AST (IU/l) in the 1st week		AST (IU/l) in the 2nd week		AST (IU/l) in the 3rd week		AST (IU/l) in the 4th week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	60	34	64	44	31	22	8	11
Mean ±SD	170.63 ±159.47	218.85 ±128.22	136.98 ±99.61	193.97 ±160.51	151.09 ±138.57	235.50 ±170.27	122.87 ±141.60	94.27 ±89.77
Range	40-852	42-589	32-418	4-676	31-578	67-617	48-467	11-332
p-value	0.009		0.054		0.016		0.563	

Table 6. Values of γ-glutamyl transferase (γ-GT) activity in Groups 1 and 2 in successive weeks of the disease

Variable	γ-GT (IU/l) in the 1st week		γ-GT (IU/l) in the 2nd week		γ-GT (IU/l) in the 3rd and 4th weeks	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	8	10	13	11	7	10
Mean ±SD	159.87 ±65.23	186.50 ±109.77	180.54 ±101.64	195.82 ±93.81	159.288 ±73.91	131.40 ±103.66
Range	80-266	41-330	60-341	40-341	38-233	41-365
p-value	0.824		0.663		0.406	

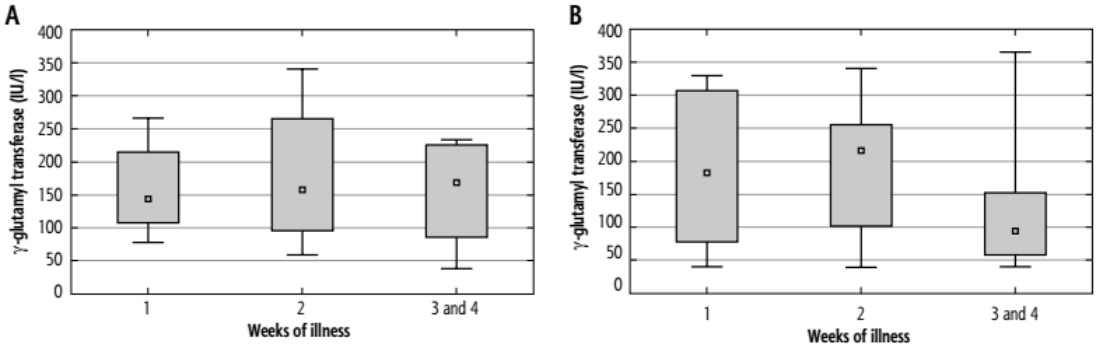

Fig. 7. Mean activity of γ-glutamyl transferase (γ-GT) (± standard deviation) in successive weeks of Epstein-Barr virus (EBV) hepatitis in patients treated with steroids (Group 1), $p = 0.697$ (**A**), and in patients not treated with steroids (Group 2), $p = 0.053$ (**B**). The Kruskal-Wallis test was used for changes over time

Among patients not receiving steroids, a peak of γ-GT activity was observed in the second week of IM. In the group of children treated with steroids, γ-GT levels showed an increasing trend with the duration of the disease. However, the differences in γ-GT levels in the successive weeks of the disease in both groups of patients were not statistically significant. Figure 7 shows the mean activity of γ-GT in successive weeks of IM in both cohorts of patients.

The median γ-GT levels between the study groups did not differ statistically significantly in any of the weeks of illness. Mean γ-GT values in successive weeks of the disease are shown in Table 6.

As described for γ-GT activity, total serum bilirubin concentration in children not treated with steroids was highest in the second week of the illness. However, these differences were not statistically significant. Among patients receiving steroids, total bilirubin remained at a comparable level throughout the course of the disease. Figure 8 shows the mean total serum bilirubin concentration in successive weeks of disease in both groups of patients.

There were no significant differences between total serum bilirubin concentrations between both study groups in the successive weeks of IM. Table 7 contains the mean values of total serum bilirubin concentrations in both groups, determined in the successive weeks of the disease.

Patients receiving steroids had a mean direct bilirubin serum concentration of 1.46 ±2.08 mg/dl (from 0.04 to 5.10). Among them, 8 (7.7%) had direct bilirubin at least 20% of total serum bilirubin concentration. The mean direct bilirubin in Group 2 (steroid-naïve)

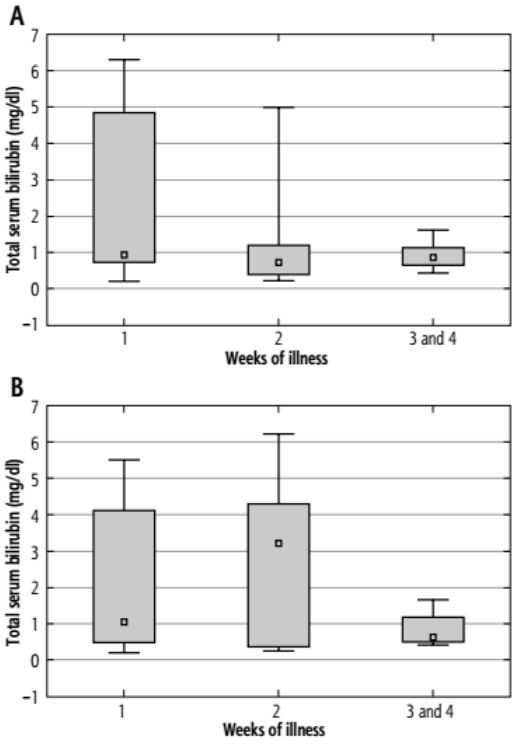

Fig. 8. Mean total serum bilirubin concentration (± standard deviation) in successive weeks of Epstein-Barr virus (EBV) hepatitis in patients treated with steroids (Group 1), $p = 0.834$ (**A**), and in patients not treated with steroids (Group 2), $p = 0.424$ (**B**). The Kruskal-Wallis test was used for changes over time

was 1.70 ±1.98 mg/dl (from 0.08 to 5.38). In 10 (16.1%) children of this group direct bilirubin accounted for at least 20% of total bilirubin.

Abdominal ultrasonography was ordered more often in patients not treated with steroids – 80.7% vs. 62.5% in Group 1 – but abnormal results were obtained in both groups in similar percentages of patients (97.2% in Group 1 vs. 95.2% in Group 2). The incidence of hepatomegaly, splenomegaly, and periportal lymphadenopathy was similar in both groups, as well. Children treated with steroids more often presented with perisplenic, intraperitoneal, and periportal fluid collection. However, gallbladder wall thickening was observed with higher frequency in patients not treated with steroids (14.5% vs. 7.7% in Group 1). The percentage of patients with particular abnormalities in the abdominal ultrasound examination in both groups is shown in Figure 9 and Table 8. Differences in the incidence of the above-described abnormalities were not statistically significant.

Treatment

The most common indications for hospitalization of children with IM are high or long-lasting fever, dehydration, and symptoms of upper respiratory tract obturation with sleep apnea. Therefore, symptomatic treatment, including the administration of antipyretics and adequate hydration, is the primary management.

The number of patients who received empirical antibiotic therapy before admission to the hospital and before the diagnosis of IM was significantly higher in Group 1 than in Group 2, and was respectively 68 (65.4%) and 23 (37.1%); $p = 0.000$. The main reason for initiating antimicrobial treatment was pharyngitis. The most commonly prescribed antibiotics were: cefuroxime axetil, amoxicillin with clavulanic acid, phenoxymethylpenicillin, azithromycin, and amoxicillin. In Group 1, antimicrobial therapy was continued after hospital admission in 43 (41.3%) patients, and in 16 (15.4%)children who had not previously been treated with antibiotics, it was started. In 12 (19.3%) patients in Group 2 antibiotic therapy was continued during hospitalization. In this group, antimicrobial treatment was started after admission to the hospital in 14 (22.6%) children. The most commonly used substance during hospitalization was cefuroxime. Eighteen patients (17.3%) in the first group and 8 (12.9%) in the second group were treated with more than one antibiotic ($p = 0.435$).

Discussion

Our retrospective study is an attempt to characterize two groups of patients hospitalized with hepatitis

Table 7. Values of total bilirubin serum concentration in Groups 1 and 2 in successive weeks of the disease

Variable	Total bilirubin (mg/dl) in the 1st week		Total bilirubin (mg/dl) in the 2nd week		Total bilirubin (mg/dl) in the 3rd and 4th weeks	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	11	11	9	6	5	4
Mean ±SD	2.13 ±2.29	1.99 ±1.92	1.46 ±1.75	2.94 ±2.36	0.92 ±0.46	0.85 ±0.57
Range	0.23-5.52	0.19-6.26	0.23-4.96	0.27-6.22	0.43-1.61	0.42-1.69
p-value	0.792		0.345		0.713	

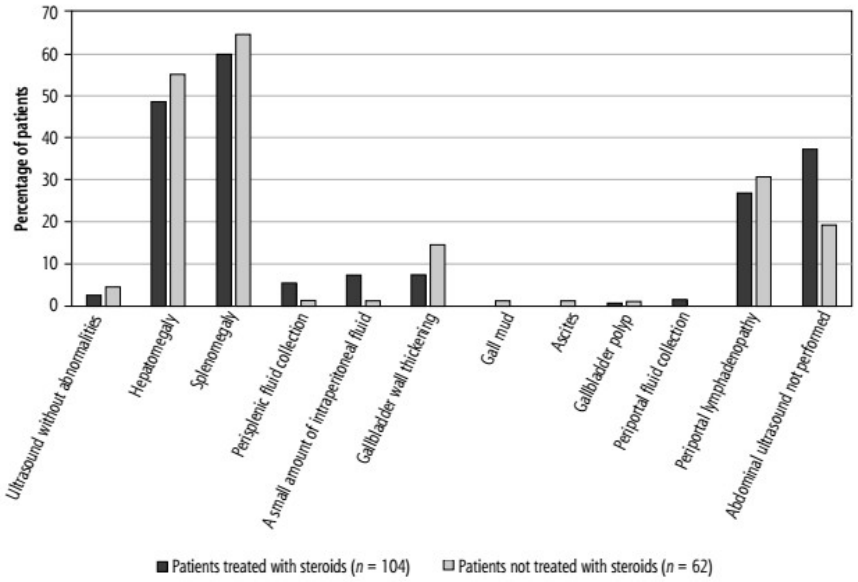

Fig. 9. Abnormalities in abdominal ultrasound in patients receiving steroids and not treated with steroids

caused by primary EBV infection. The first group includes patients treated with steroids due to complications of infectious mononucleosis (mainly upper respiratory tract obturation), and the second group consists of children not receiving the above therapy. In both groups, there is a noticeable predominance of persons in preschool and early school age (4-8 years old) and adolescents (14-18 years old), which is characteristic of the epidemiology of IM [18-20]. However, the above-described distribution of age groups is more pronounced among patients treated with steroids. Both groups are also characterized by a similar gender distribution: the percentage of males was 40.1% and 41.9%, respectively, in Groups 1 and 2.

The rich symptomatology of IM causes patients to present with a diverse constellation of symptoms and possible complications [21, 22]. There were also some differences between the two groups we studied. When analyzing the course of the disease, we found that patients in the steroid-treated group stayed longer in the hospital, and more often presented the classic features of IM with fever, which was the most common clinical symptom in them and lasted longer than in Group 2. They were also more likely to have hepatosplenomegaly on palpation, although the presence of other clinical signs suggestive of hepatitis (with features of cholestasis in some cases) was not significantly different between the two groups. Complications other than upper airway obturation occurred with similar frequency in both cohorts.

Table 8. Incidence of particular abnormalities in abdominal ultrasound examination in the studied groups of patients

Variable	Group 1, *n* = 104 No. of patients (%)	Group 2, *n* = 62 No. of patients (%)
Abdominal ultrasound without abnormalities	3 (2.8)	3 (4.8)
Hepatomegaly	51 (49.0)	34 (54.8)
Splenomegaly	62 (59.6)	40 (64.5)
Perisplenic fluid collection	6 (5.7)	1 (1.6)
A small amount of intraperitoneal fluid	8 (7.7)	1 (1.6)
Gallbladder wall thickening	8 (7.7)	9 (14.5)
Gall mud	0 (0.0)	1 (1.6)
Ascites	0 (0.0)	1 (1.6)
Gallbladder polyp	1 (0.9)	1 (1.6)
Periportal fluid collection	2 (1.9)	0 (0.0)
Periportal lymphadenopathy	28 (26.9)	19 (30.6)
Abdominal ultrasound not performed	39 (37.5)	12 (19.3)

As regards the laboratory test results other than those evaluating liver function, statistically significant differences occurred only in the percentage of neutrophils in the white blood cell smear and CRP concen-

tration. Both of these parameters were higher in patients who received steroid therapy.

The available literature provides an analysis of the laboratory test results assessing liver function in the course of IM [8, 9, 23]. However, it is difficult to find such research in the context of steroid therapy used in these patients. Analysis of the course of ALT and AST activity during the disease showed higher activity of both enzymes in the first, second, and third weeks of IM in patients not treated with steroids. However, statistically significant differences were observed only in the first week for ALT and in the first and third for AST. In an analysis involving the variability of enzyme activity over time, levels of both enzymes showed peaks at the first and third weeks of the disease in steroid-naïve patients. However, statistically significant differences were seen only for AST activity. Our observations are consistent with the results of other available studies [23]. These results may indicate greater severity of hepatitis (defined as increased ALT and AST levels) in patients with less pronounced upper respiratory tract IM symptoms: pharyngitis and tonsillitis, cervical lymphadenopathy, and consequent upper airway obturation. Despite the observed lower activity of ALT and AST in the first three weeks of the disease in patients treated with steroids, in the fourth week (usually after the end of steroid therapy) the levels of both enzymes were higher than in patients not receiving steroids. Thus, the anti-inflammatory effect of temporarily administered glucocorticosteroids (in doses appropriate for the treatment of the complications of IM) might be insufficient to achieve a sustained reduction in hepatic inflammation. However, this conclusion would require confirmation in further (more detailed) research.

Cholestasis is a possible complication of EBV-associated hepatitis [24-26]. Concerning the results of laboratory tests useful in the diagnosis of cholestasis (γ-GT and total serum bilirubin concentration with fractions), no statistically significant differences were obtained between the study groups. γ-GT activity was higher in steroid-naïve patients in the first two weeks of illness. Among these patients, peak γ-GT levels were observed in the second week of IM, as was the case for total bilirubin. Direct bilirubin of at least 20% of total bilirubin was observed more frequently in steroid-naïve patients (16.1%) than in children receiving steroids (7.7%).

Abdominal ultrasonography revealed perisplenic, intraperitoneal, and periportal fluid collection more often in patients receiving steroids, but gallbladder wall thickening was described with higher frequency among steroid-naïve patients. However, the above differences did not show statistical significance.

Patients who required steroids significantly more often started empiric antibiotic therapy before admission to the hospital. They were also more likely to continue antimicrobial treatment during hospitalization. Available literature data also indicate frequent administration of antibiotics in patients with upper respiratory tract complications of IM [27-29]. However, in our patients, the above therapy was implemented only after admission to the hospital in a larger proportion of steroid-naïve patients.

An undoubted limitation of our study is its retrospective nature and the fact that not all patients in both study groups underwent all laboratory tests that would allow for a comprehensive assessment of liver function and abdominal ultrasound. However, our analysis concerns a fairly large cohort of patients. Literature data related to this subject are limited, and most studies to date have been conducted on much smaller groups [4, 30, 31]. It should be noted that the doses of corticosteroids used in our patients were adjusted to treat airway obturation. Perhaps administration of higher doses would result in a stronger anti-inflammatory effect in hepatitis.

In conclusion, our study suggests that the two analyzed groups may differ in the course of hepatitis in primary EBV infection, especially in the first weeks of IM, when the laboratory features of hepatitis were more pronounced in children not requiring treatment with steroids. However, there was a noticeable trend for higher values of liver parameters at the end of the disease (suggestive of delayed resolution of hepatitis) in patients receiving steroids, but without statistical significance. A possible explanation for the above phenomenon is potentially delayed viral clearance caused by steroid therapy [3]. Cessation of steroid treatment and the disappearance of their anti-inflammatory effect could increase the activity of liver enzymes.

Institutional Review Board Statement

Ethical review and approval were waived for this study because the study was retrospective, non-interventional, and based on data collected in the department's database. Therefore, it does not require approval of the Ethics Committee. Due to the retrospective nature of the present study, written consent from participants was not necessary. The patients' data were protected according to the European Union General Data Protection Regulation.

Disclosure

The authors declare no conflict of interest.

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Publikacja nr 3.

Rutkowska M, Pokorska-Śpiewak M.

The impact of acute CMV coinfection on the course of EBV hepatitis in children.

Clin Exp HEPATOL. 2025

Clinical and Experimental HEPATOLOGY

Clin Exp HEPATOL 2025; 11, 3: 1–10
DOI: https://doi.org/10.5114/ceh.2025.154284
Received: 04.03.2025, **Accepted:** 18.05.2025, **Published:** 2025

Original paper

The impact of acute CMV coinfection on the course of EBV hepatitis in children

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Abstract

Aim of the study: Hepatitis occurs in 50-90% of patients hospitalized with primary Epstein-Barr virus (EBV) infection. Dual serological positivity for EBV and cytomegalovirus (CMV) may occur. Our retrospective study aimed to compare the clinical course of hepatitis between children with EBV monoinfection (Group 1) and those with EBV and CMV dual positivity (Group 2).

Material and methods: In total, 244 patients were recruited. Eighty (32.79%) of them belonged to Group 2. The diagnosis of EBV and CMV infection was confirmed by positive viral capsid antigen (VCA) antibody testing and serum CMV IgM antibodies.

Results: Clinical symptoms suggesting cholestasis occurred significantly more often in Group 2: nausea or vomiting in 35 (43.21%) vs. 45 (27.44%) in Group 1, $p = 0.010$ (OR = 2.01 [95% CI: 1.15-3.51]); abdominal pain in 37 (45.67%) vs. 52 (31.71%), $p = 0.042$ (OR = 1.81 [95% CI: 1.05-3.13]); dark urine in 12 (14.81%) vs. 8 (4.88%), $p = 0.006$ (OR = 3.39 [95% CI: 1.33-8.67]). The alanine aminotransferase (ALT) activity was higher in Group 2 during the first week of the disease, although the difference was not statistically significant ($p = 0.083$). Aspartate aminotransferase (AST) activity was significantly higher in Group 2 only in the first week of the disease ($p = 0.023$). Abnormal abdominal ultrasound results occurred more often in Group 2: 100% of patients vs. 66.46% in Group 1 ($p = 0.045$).

Conclusions: In Group 2, we observed more pronounced clinical symptoms of hepatitis and more frequently abnormal ultrasound images of the liver and bile ducts.

Key words: Epstein-Barr Virus, cytomegalovirus, liver aminotransferases, children, viral hepatitis.

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Introduction

Infectious mononucleosis (IM) is a common disease in children and adolescents that most often results from acute Epstein-Barr virus (EBV) infection. In early childhood, it is usually asymptomatic or mildly symptomatic, while 30-50% of teenagers and young adults develop a broad variety of clinical symptoms of IM [1]. The classic triad of symptoms, including fever, sore throat, and generalized lymphadenopathy, is often accompanied by fatigue, mild headache, and hepatosplenomegaly.

The available data from the literature show that the percentage of children with hepatitis (usually asymptomatically elevated transaminases) in the course of EBV infection ranges from 50% (Polish study) to 80-90% (studies conducted in England and Greece) [2-4]. These data concern patients hospitalized due to IM. The activity of liver transaminases in serum most often does not exceed two or three times the upper limit of normal [5]. Although cholestatic hepatitis is rare and usually reported in adults, it can also develop in children; in a Polish study, it occurred in 10.8% of pediatric patients with hepatitis due to primary EBV infection [2, 6].

In approximately 10% of patients with infectious mononucleosis-like syndrome, the etiological factor is not EBV and may include cytomegalovirus (CMV), primary hepatotropic viruses, human immunodeficiency virus (HIV) – acute retroviral disease, or adenovirus [7]. Primary EBV and CMV infections in immunocompetent individuals cause similar clinical and laboratory symptoms. Studies report a more severe course of the disease in patients with dual-positivity for EBV and CMV than in those with positive only anti-VCA IgM for EBV [8]. Moreover, according to some authors, CMV infection may be associated with more severe hepatitis [9, 10].

Acute EBV and CMV coinfection confirmed by positive serological test results (immunoglobulin M [IgM]) is considered by many researchers and clinicians to be a false-positive finding due to the cross-reaction of serum antibodies [11-14].

From April 2023 to May 2024, we observed an increase in the number of patients hospitalized in our department with hepatitis in the course of IM and positive serological test results for EBV and CMV compared to the previous years.

The present study aimed to compare the course (in terms of clinical symptoms and laboratory and imaging test results) of hepatitis in pediatric patients with positive anti-VCA IgM for EBV and in those with dual serological positivity for EBV and CMV, and to identify the differences between the cohorts.

Material and methods

Patients

Our retrospective, single-center study was based on the analysis of the clinical data of patients aged 0-18 years who were hospitalized for acute hepatitis due to EBV infection (with or without positive IgM for CMV) at the Department of Children's Infectious Diseases, Medical University of Warsaw, Regional Hospital of Infectious Diseases in Warsaw, between November 2017 and May 2024.

We established the following exclusion criteria:

- coexisting viral hepatitis (hepatitis A virus [HAV], hepatitis B virus [HBV], hepatitis C virus [HCV]),
- preexisting human immunodeficiency virus (HIV) infection,
- other liver diseases (e.g., metabolic or autoimmune) associated with impaired liver function,
- hepatotoxic drug therapy due to underlying chronic diseases and other causes of toxic liver damage,
- chronic diseases affecting the host's immune response (e.g., immunodeficiencies, severe hemoglobinopathies, cancer).

Methods

We considered the onset of the disease to be the first day of symptoms typical for infectious mononucleosis syndrome (fever, lymphadenopathy, sore throat). Some patients experienced isolated gastrointestinal symptoms, jaundice, hepatosplenomegaly, or increased liver aminotransferase activity. We confirmed EBV infection using a serological test detecting IgM antibodies to the EBV viral capsid antigen (anti-EBV VCA IgM, chemiluminescence immunoassay – CLIA test system, LIAISON EBV IgM, DiaSorin S.p.A., Italy) as a marker which does not occur in chronic disease. Similarly, the diagnosis of acute CMV infection was based on the detection of immunoglobulin M antibodies to CMV in serum or plasma samples (chemiluminescence immunoassay – CLIA test system, LIAISON CMV IgM, DiaSorin S.p.A., Italy). Both tests are routinely performed in patients suspected of having infectious mononucleosis. Depending on clinical indications, tests for other hepatotropic viruses were ordered.

The laboratory indicator of ongoing hepatitis was increased over the upper limit of normal (concerning age and sex references) activity of serum alanine aminotransferase (ALT) [15]. Additionally, we assessed the activity of the serum aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), and bilirubin concentrations in the fractions. AST and γ-GT values were related to age- and sex-specific norms [15]. Other laboratory tests included a full blood count with differential and C-reactive protein (CRP) levels. We defined leukocytosis based on age and sex standards [16]. Thrombocytopenia was defined as a platelet count of less than 100,000 per mm^3.

During the abdominal ultrasound examination (which was performed in the vast majority of patients), we paid special attention to the size of the liver, spleen, and lymph nodes in the liver hilum, as well as to the pathology of the gallbladder and bile ducts. Splenomegaly and hepatomegaly on ultrasound examination were diagnosed when the longitudinal dimension of these organs exceeded the 97.5^{th} percentile for age [17].

In both study groups, we identified patients with a more severe course of the disease who met at least one of the following criteria:

- at least three of the following signs or symptoms: nausea/vomiting, darkening of urine (the color of urine darker than in a healthy state), discoloration of stool, jaundice, pruritus, abdominal pain or

- at least one laboratory abnormality of the following: ALT activity equal to or greater than five times the upper limit of normal for sex and age, increased γ-GT activity in serum, direct bilirubin concentration equal to or greater than 20% of total serum bilirubin concentration or
- signs of inflammation in the gallbladder/biliary tract on abdominal ultrasound.

Statistical analysis

We performed a statistical analysis of the collected medical records using STATISTICA 13.3 (StatSoft, Kraków, Poland). The data are presented as the means (standard deviations; SDs) unless otherwise indicated. The comparison of clinical features between study cohorts was performed using Fisher's exact test or Pearson's chi-square test (for categorical variables) and the Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables, such as laboratory findings, the duration of signs and symptoms, and the length of hospitalization. None of the studied groups showed a normal distribution concerning the analyzed variables. The level of statistical significance was set at $p < 0.05$.

Results

Demographic and clinical characteristics

We performed a retrospective analysis of the medical records of 253 pediatric patients who were hospitalized due to hepatitis with increased ALT activity in the course of EBV infection with or without positive IgM for CMV. Nine patients (3.55%) were excluded from the study because of underlying diseases associated with impaired liver function or potentially hepatotoxic drug therapy. For the study, we recruited 244 children and adolescents aged 0-18 years and divided them into two groups. The first cohort consisted of 164 patients (67.21%) with hepatitis during EBV infection (the male-to-female ratio was 1 : 1.41). In the second group of 80 (32.79%) children with increased ALT activity due to EBV infection with positive IgM for CMV, the male-to-female ratio was 1 : 1.86. The enrollment of patients in the study is presented in the flowchart (Fig. 1).

The age distributions in both cohorts were similar: 12.02 ±5.00 years in the first group and 11.92 ±5.33 years in the second group ($p = 0.851$), with a predominance of children aged 3-8 and 13-18 years as typical epidemiological features of IM.

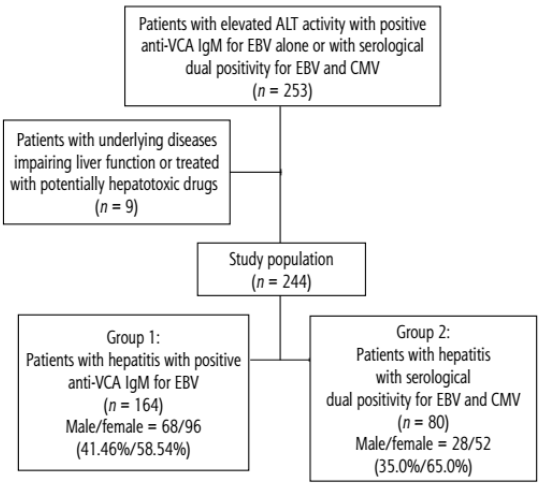
Children in the second group more often suffered from chronic diseases (29; 35.80%) than did patients in the first group (42; 25.61%), although this difference was not statistically significant ($p = 0.085$). The most common were allergic diseases (bronchial asthma, inhalant or food allergy, atopic dermatitis), neuropsychiatric disorders (autism, depressive disorders, recurrent headaches), and adenoid hypertrophy. The incidence of particular disease entities did not differ significantly between the groups. The drugs used to treat the above conditions did not affect liver function.

Clinical course and complications

The analysis of the course of the disease did not reveal any significant differences between the study groups in terms of the duration of signs and symptoms or hospitalization. These data are presented in Table 1.

A detailed analysis of the prevalence of particular symptoms and signs of IM revealed significant differences concerning the following:

- pharyngitis with tonsillar exudate was more common among patients with isolated EBV infection, 148 (90.24%) vs. 65 (80.25%) in patients with EBV and CMV dual positivity; $p = 0.047$;
- nausea or vomiting occurred more often in the second study group: 35 (43.21%) vs. 45 (27.44%) in the first group; $p = 0.010$ (OR = 2.01 [95% CI: 1.15-3.51]);
- abdominal pain was reported more frequently in children in Group 2 (37 (45.67%) than in those in Group 1 (52 (31.71%)); $p = 0.042$ (OR = 1.81 [95% CI: 1.05-3.13]);
- dark urine was observed in 12 (14.81%) individuals with EBV and CMV serological dual positivity and



ALT – alanine aminotransferase, CMV – cytomegalovirus, EBV – Epstein-Barr virus

Fig. 1. Study flow diagram

Table 1. Differences in demographic and clinical features between the study groups

Variable	Group 1 (n = 164)	Group 2 (n = 80)	p-value
	Mean ±SD (range)		
Age (years)	12.02 ±5.00 (1.50-18.00)	11.92 ±5.33 (1.50-17.80)	0.850
Sex (male/female)	68/96	28/52	0.413
Duration of signs and symptoms (days)	14.46 ±5.36 (4.00-30.00)	15.06 ±5.77 (7.00-32.00)	0.613
Days of hospitalization	4.74 ±2.18 (1.00-14.00)	4.82 ±2.13 (1.00-11.00)	0.727
Fever duration (days)	6.91 ±4.83 (0.00-21.00)	7.64 ±5.87 (0.00-24.00)	0.467

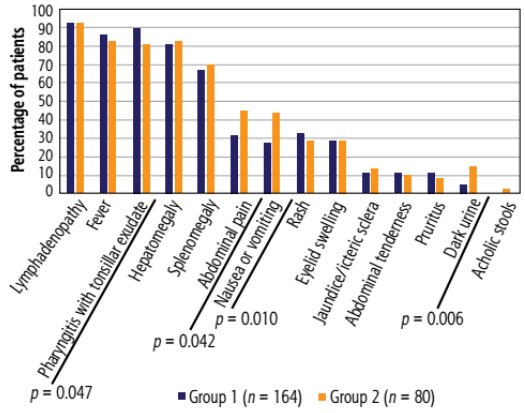


Fig. 2. Frequency of infectious mononucleosis (IM) symptoms in Group 1 and Group 2

in 8 (4.88%) with EBV monoinfection; p = 0.006 (OR = 3.39 [95% CI: 1.33-8.67]).

The frequency of other symptoms of mononucleosis syndrome did not differ significantly between the study groups. Figure 2 shows the frequency of IM symptoms in both cohorts.

Complications (except those related to the liver and biliary tract) were diagnosed in 24 (14.63%) patients in the first group and 13 (16.25%) in the second group (without any significant difference). The most frequently observed sequelae were as follows (number and percentage of patients in Groups 1 and 2, respectively): upper respiratory tract obturation: 68 (41.46%) vs. 25 (31.25%); rash after amoxicillin administration: 27 (16.46%) vs. 8 (10%); thrombocytopenia (with or without purpura): 4 (2.44%) vs. 3 (3.75%); and urticaria: 2 (1.22%) only in the first group.

The frequency of infectious complications (or co-occurring infections) was significantly higher in children in the first group: 80 (48.78%) than in those in the second group: 28 (34.57%, p = 0.036). The most frequently diagnosed infections were as follows (in Groups 1 and 2): bacterial pharyngitis (usually caused by *Streptococcus pyogenes*): 54 (32.93%) vs. 16 (20.00%); acute otitis media: 4 (2.44%) vs. 3 (3.75%); oral candidiasis ("thrush stomatitis"): 3 (1.83%) in the first group; and bacterial pneumonia: 2 (1.22%) vs. 1 (1.25%). However, only scarlet fever was diagnosed significantly more often in the first Group: 2 (1.22%) vs. 2 (2.50%), p = 0.027.

Laboratory and imaging test results

When we analyzed the values of the inflammation parameters, we detected significant differences in the CRP serum concentration – 21.93 ±16.54 mg/dl in Group 1 vs. 15.15 ±12.85 mg/dl in Group 2 (p = 0.001) – and the percentage of neutrophils in the manual white blood cell smear: 32.07 ±13.25% in the first group vs. 25.33 ±12.35% in the second group (p = 0.000). Leukocytosis occurred at a comparable frequency in both study groups: 119 (72.56%) patients in the first group and 59 (74.68%) children in the second group. Lactate dehydrogenase (LDH) level was higher, though not significantly, in children with EBV and CMV dual positivity (p = 0.089). Table 2 presents the comparison of laboratory test results between the study groups.

The serum activity of ALT in the successive weeks of the disease in both study groups is presented in Figure 3. The analysis conducted over time revealed no significant differences between the cohorts. In patients positive for EBV and CMV, the values were higher throughout the disease than in children with isolated EBV infection, but without reaching statistical significance (in the first week, p = 0.083). The values discussed are presented in Table 3.

Aspartate aminotransferase activity in the serum did not significantly differ between successive weeks of

disease in either study group (Fig. 4). In the first group, AST values were highest in the second week of illness, with a decreasing trend in the third and fourth weeks. In the second group, the highest AST levels were observed during the first and fourth weeks. However, AST activity was significantly higher in the second group only in the first week ($p = 0.023$). The values of AST in the serum in successive weeks of illness in both study groups are presented in Table 4.

Analysis of γ-GT revealed the highest activity in the first group in the second week of the disease and in the second group in the first week (Fig. 5). In both cohorts, the differences between the successive weeks were not statistically significant (although in Group 1 the *p*-value reached 0.082). In the second week the activity of this enzyme showed a non-significant difference between groups ($p = 0.091$). The values of γ-GT activity in the serum in successive weeks of illness in both study groups are presented in Table 5.

The concentration of total bilirubin in the serum in both groups was the highest in the first week of the disease and was similar, as shown in Figure 6. The greatest difference was observed in the second week. The above data are presented in Table 6. The percentage of children whose direct bilirubin concentration exceeded 20% of total serum bilirubin in the first group was 80.95%, and in the second group it was 78.26% (this difference was not significant).

For all patients in both groups, the Quick index values were within normal limits.

All the children underwent abdominal ultrasound examination, with significantly more abnormal results (100% of patients) in the second group than in the first group (66.46%), $p = 0.045$. However, the frequency of particular abnormalities on abdominal ultrasound did not differ significantly between the study groups, although they occurred more often in children with EBV and CMV dual positivity: hepatomegaly in 71 (43.29%) patients in the first group vs. 41 (51.25%) in the second group ($p = 0.532$); enlargement of the lymph nodes in the liver hilum in 63 (38.41%) in Group 1 and 40 (50.00%) in Group 2 ($p = 0.921$); and abnormalities of the gallbladder and bile ducts in 18 (10.97%) children in the first group vs. 16 (20.00%) in the second group ($p = 0.297$). Abnormalities of the gallbladder and biliary tract included thickening of the gallbladder wall, dilatation of the bile ducts, and accumulation of fluid in the hilum of the liver and around the gallbladder.

Holistic assessment of the course of hepatitis

Our criteria for a more severe course of the disease were met by 96 (58.54%) children from the first group and by 60 (74.07%) patients from the second group, $p = 0.018$ (OR = 2.02 [95% CI: 1.13-3.64]).

Treatment

All study patients received supportive therapy for fever, dehydration, respiratory tract obturation, or severe sore throat.

Table 2. Comparison of laboratory test results between study groups

Variable	Group 1 (*n* = 164)	Group 2 (*n* = 80)	*p*-value
	Mean ± SD (range)		
White blood cell count (cells/μl)[a]	14.41 ±6.40 (4.47-49.00)	15.03 ±5.58 (5.10-33.90)	0.136
Neutrophils (%)[a]	32.07 ±13.25 (5.00-77.00)	25.33 ±12.35 (2.00-57.00)	0.000
Atypical lymphocytes (%)[a]	25.71 ±14.56 (0.00-70.00)	28.27 ±15.18 (6.00-80.00)	0.276
CRP (mg/dl)[b]	21.93 ±16.54 (0.41-75.00)	15.15 ±12.85 (5.00-58.00)	0.001
PCT (ng/ml)[c]	1.98 ±7.04 (0.05-37.00)	0.60 ±0.72 (0.08-3.44)	0.787
Platelets (cells/μl)[d]	209.97 ±67.17 (9.80-4.55)	205.87 ±74.49 (9.7-3.66)	0.658
LDH (U/l)[e]	686.37 ±238.60 (413.00-1427.00)	842.67 ±283.14 (400-1350)	0.089

[a] White blood cell smears, including the percentages of lymphocytes, neutrophils, and atypical lymphocytes, were assessed in 160 patients in Group 1 and 78 children in Group 2; [b] CRP (C-reactive protein) was evaluated in 159 patients in Group 1 and 78 children in Group 2; [c] PCT (procalcitonin) was assessed in 27 patients in Group 1 and 28 children in Group 2; [d] Platelets were evaluated in 163 patients in Group 1 and 79 children in Group 2; [e] LDH (lactate dehydrogenase) was evaluated in 19 patients in Group 1 and 15 children in Group 2

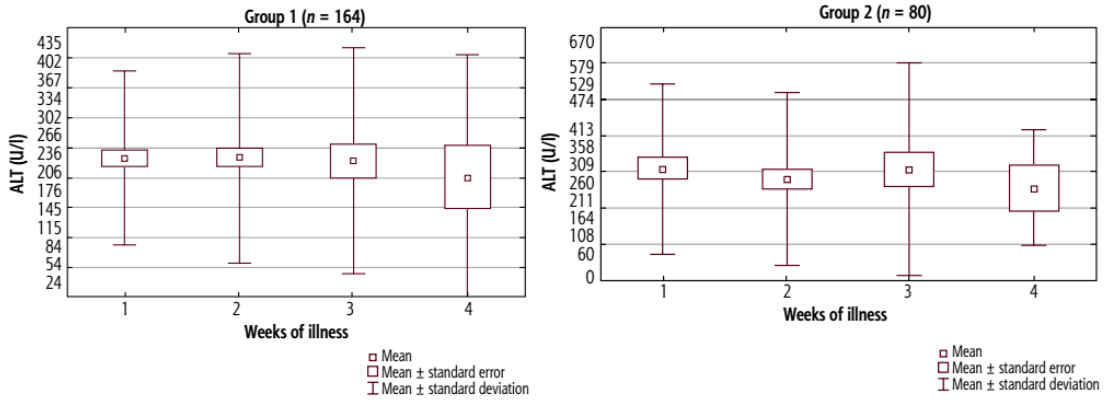


Fig. 3. Mean activity (±SD) of alanine-aminotransferase (ALT) in successive weeks of hepatitis in Group 1, $p = 0.309$, and Group 2, $p = 0.864$. The Kruskal-Wallis test was used for changes over time

Table 3. Values of alanine-aminotransferase (ALT) activity in serum of patients in Groups 1 and 2 during successive weeks of the disease

Variable	ALT (IU/L) in 1st week		ALT (IU/L) in 2nd week		ALT (IU/L) in 3rd week		ALT (IU/L) in 4th week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	113	57	120	69	45	39	15	6
Mean ±SD	232.65 ±144.81	310.07 ±238.26	233.15 ±174.68	281.88 ±238.59	227.22 ±188.34	306.56 ±292.16	200.40 ±204.50	255.00 ±160.46
Range	39.00-725.00	22.00-1082.00	42.00-1105.00	32.00-1234.00	42.00-791.00	23.00-1560.00	24.00-814.00	64.00-474.00
p-value	0.083		0.370		0.187		0.330	

Empirical antibiotic therapy (most often due to pharyngitis) had been implemented before hospitalization (and final diagnosis) in 90 (54.87%) patients from the first group and 34 (41.98%) from the second group ($p = 0.116$). Amoxicillin was most frequently prescribed: 41 (25.00%) patients with EBV monoinfection vs. 15 (18.52%) children with EBV and CMV dual-positivity, $p = 0.275$. Twenty-seven patients (16.46%) from the first group and eight (9.87%) from the second group developed an allergic-toxic rash after amoxicillin ($p = 0.176$). Cefuroxime axetil was the second most commonly used antibiotic.

After admission to the hospital (and IM diagnosis), 83 (50.61%) children from the first group required antibiotic therapy compared to only 27 (33.33%) patients from the second group ($p = 0.011$). The most common indication was pharyngitis. Cefuroxime was the most frequently used agent: in 61 (73.49%) children from the first group and 18 (66.66%) from the second group. Five (6.02%) children with EBV monoinfection and three (1.11%) with EBV and CMV dual positivity required therapy with two antibiotics. The second antimicrobial agent most frequently used was clindamycin (due to inflammation of the cervical lymph nodes).

All patients fully recovered without any chronic complications.

Discussion

Our retrospective study aimed to compare the clinical course of hepatitis between pediatric patients with EBV monoinfection (Group 1) and those with EBV and CMV dual positivity (Group 2). The percentage of children belonging to the second group was 32.79% of all patients, which was comparable to that reported in other studies (20-42%) [1, 18, 19].

This study covers the period from November 2017 to May 2024. In our previous study, we described the course of hepatitis caused by primary EBV infection in children hospitalized from August 2017 to March 2023 [2]. The number of patients with hepatitis due to EBV mono-infection is comparable in both studies. From April 2023 to May 2024, the majority of children with hepatitis associated with IM syndrome had positive serological test results for EBV and CMV.

The studies conducted thus far among patients with IM and hepatic involvement usually showed that positive IgM for CMV was the effect of the antigenic cross-reactivity related to the EBV infection [1, 12, 13, 20]. Most of these studies were conducted in adult patients. The phenomenon of positive antibodies against both viruses can be explained by the polyclonal production of antibodies against other viruses from the

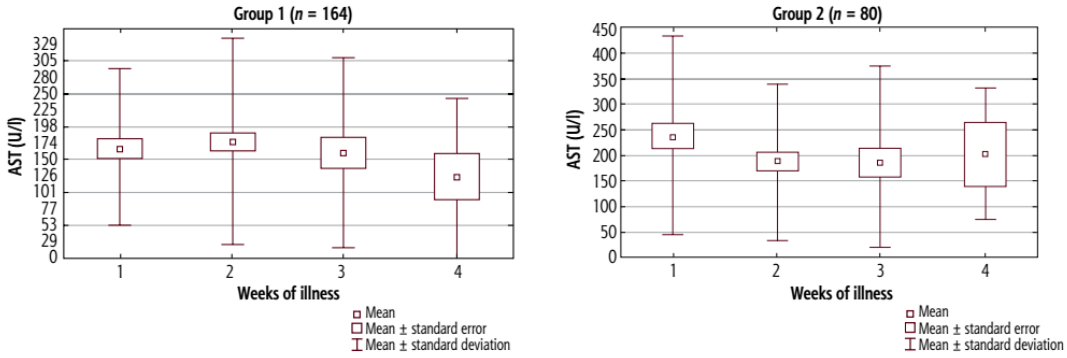


Fig. 4. Mean activity (±SD) of aspartate-aminotransferase (AST) in successive weeks of hepatitis in Group 1, $p = 0.107$, and Group 2, $p = 0.116$. The Kruskal-Wallis test was used for changes over time

Table 4. Aspartate-aminotransferase (AST) activity in serum of patients in Groups 1 and 2 during successive weeks of disease

Variable	AST (IU/l) in 1st week		AST (IU/l) in 2nd week		AST (IU/l) in 3rd week		AST (IU/l) in 4th week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	100	57	119	64	41	39	15	4
Mean ±SD	170.37 ±118.17	238.40 ±192.52	177.55 ±157.76	187.26 ±151.37	161.27 ±144.12	186.43 ±187.98	121.00 ±123.42	202.75 ±125.78
Range	4.00-615.00	26.00-1082.00	32.00-852.00	13.00-709.00	31.00-617.00	29.00-897.00	11.00-467.00	52.00-354.00
p-value	0.023		0.455		0.813		0.177	

Herpesviridae family (e.g., CMV, varicella-zoster virus, human herpes virus 6) caused by acute EBV infection [21]. According to the second theory, EBV infection causes transient immunosuppression, which favors the reactivation of latent CMV infection [20].

Some authors have suggested that liver involvement in EBV infection may be the cause of double positivity for EBV and CMV by stimulating the production of antibodies [1].

In our study, the age distribution in both groups was similar, which is inconsistent with the observations of other authors, indicating more frequent coexistence of positive results for EBV and CMV among younger children [1, 18].

Complaints and symptoms that may indicate the development of cholestasis were significantly more frequently reported among children with positive serological test results for both viruses (Group 2). Nausea, vomiting, and abdominal pain occurred approximately twice as often as they did in the first group, and darkening of urine occurred more than three times as often.

Pharyngitis and tonsillitis (usually due to *Streptococcus pyogenes*) were observed significantly more often among patients with positive serological testing only for EBV. Other concomitant infections were also diagnosed more frequently in the first group, although this difference was not statistically significant. The above observations were reflected in a significantly higher CRP concentration in the serum and the percentage of neutrophils in the manual smear of white blood cells.

The difference in the incidence of scarlet fever between study groups was statistically significant, but this result should be questioned due to the small number of patients with this condition in both cohorts.

Alanine aminotransferase and AST activities were greater among children with positive EBV and CMV antibodies throughout the disease course (at the 4-week follow-up), but a significant difference was observed for both transaminases only in the first week. Similar results were reported by Sohn *et al.* [1]. The γ-GT activity and total serum bilirubin concentration did not differ significantly between the study groups. The percentage of children with direct bilirubin concentrations exceeding 20% of total serum bilirubin was also comparable. No disorders of the synthetic function of the liver were found in any of the patients; the Quick index was normal.

In the second group, abnormal abdominal ultrasound results were obtained significantly more often. Particular abnormalities (hepatomegaly, enlarged lymph nodes in the liver hilum, thickening of the gall-

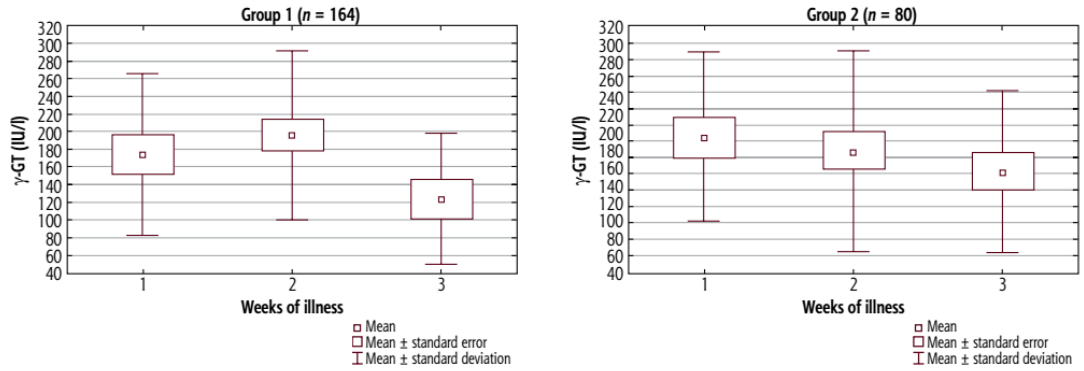


Fig. 5. Mean activity (±SD) of γ-glutamyl transferase (γ-GT) in successive weeks of hepatitis in Group 1, $p = 0.082$, and Group 2, $p = 0.308$. The Kruskal-Wallis test was used for changes over time

Table 5. Values of γ-glutamyl transferase (γ-GT) activity in serum of patients in Groups 1 and 2 during successive weeks of disease

Variable	γ-GT (IU/l) in 1st week		γ-GT (IU/l) in 2nd week		γ-GT (IU/l) in 3rd week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	18	19	27	29	13	18
Mean ±SD	174.66 ±91.19	185.10 ±103.34	196.18 ±95.89	168.86 ±122.53	125.31 ±73.77	142.83 ±98.11
Range	41.00-330.00	31.00-403.00	40.00-365.00	30.00-556.00	38.00-257.00	23.00-357.00
p-value	0.879		0.091		0.810	

bladder wall, or accumulation of fluid around the gallbladder) also occurred more frequently in this group, although the differences were no longer statistically significant.

Children with EBV and CMV dual positivity met the criteria (created for our study), indicating a more severe course of hepatitis (than usually observed in IM) more than twice as often as did patients from the first group.

In children with EBV monoinfection, antibiotic therapy after hospital admission was used significantly more often (usually due to acute bacterial pharyngitis). The rate of prescription of antibiotics in the first group reached 50.61%, which is consistent with the results of studies by other authors [10, 18]. In children diagnosed with bacterial pharyngitis, the McIsaac clinical criteria were met, and the *S. pyogenes* rapid test was performed. Patients with streptococcal pharyngitis received cefuroxime after hospital admission (although it is not the first-line therapy), because EBV infection may predispose to bacterial superinfections of etiology other than *S. pyogenes* (bacterial superinfection of mixed etiology).

The strength of this work is the large number of patients included in our study.

An inherent limitation of this study is its retrospective nature, which is based on medical records, and the absence of some data in a few cases. Only patients who required hospitalization were evaluated. Another limitation is the lack of confirmation of acute CMV infection with additional tests, such as polymerase chain reaction (PCR), IgG seroconversion, or detection of CMV antigen; therefore, a false-positive result due to antibody cross-reaction cannot be ruled out.

In conclusion, the results of our study highlighted several characteristics of children with dual positivity for EBV and CMV compared to those who were positive for EBV only. In children with dual positivity for EBV and CMV, we observed more pronounced clinical symptoms of hepatitis and more frequently abnormal ultrasound images of the liver and bile ducts. These differences may reflect true EBV and CMV coinfection or may result from individual variability in immune system function.

Nevertheless, the above uncertainties may be clarified in subsequent prospective studies using broader molecular diagnostic methods.

Disclosures

This research received no external funding.
Institutional review board statement: Not applicable.
The authors declare no conflict of interest.

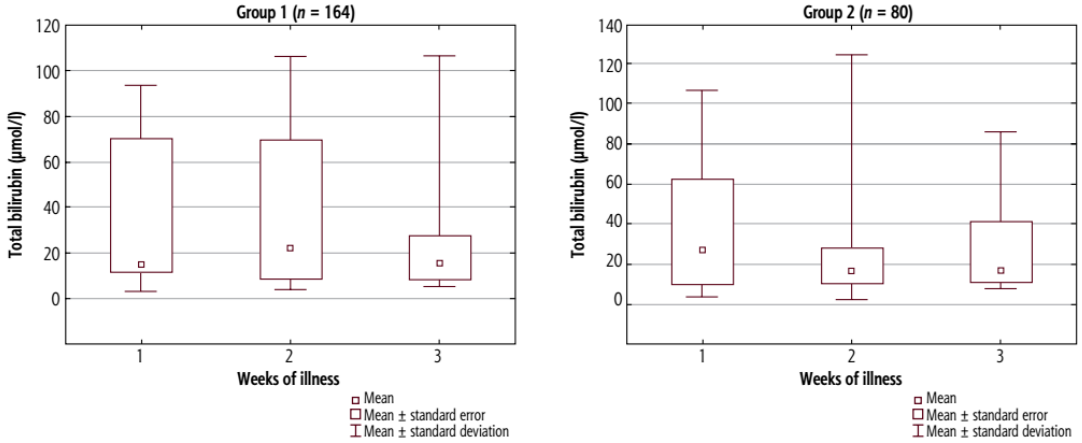


Fig. 6. Mean total bilirubin serum concentration (±SD) in successive weeks of hepatitis in Group 1, $p = 0.698$, and Group 2, $p = 0.769$. The Kruskal-Wallis test was used for changes over time

Table 6. Serum total bilirubin concentrations in Groups 1 and 2 during successive weeks of the disease

Variable	Total bilirubin (µmol/l) in 1st week		Total bilirubin (µmol/l) in 2nd week		Total bilirubin (µmol/l) in 3rd week	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
No. of patients	16	17	26	19	25	4
Mean ±SD	35.44 ±36.26	35.85 ±33.61	35.02 ±31.08	26.93 ±34.22	25.68 ±25.65	26.52 ±21.93
Range	3.40-107.00	4.00-106.93	4.00-106.30	1.80-125.00	4.70-107.00	7.30-54.50
p-value	0.673		0.311		0.949	

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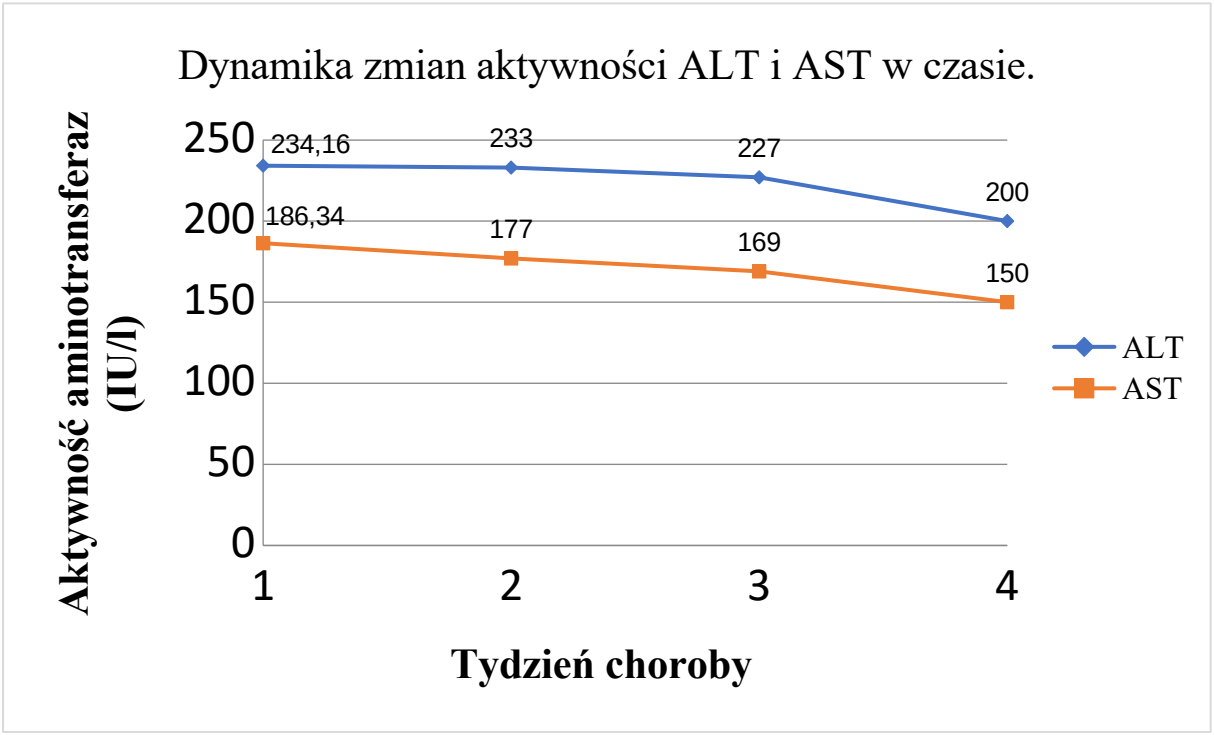
Podsumowanie łączące wyniki zawarte w cyklu publikacji

W **Publikacji nr 1.** wykazano, że zapalenie wątroby występowało u 199 spośród 415 (47,95%) dzieci hospitalizowanych z powodu mononukleozy zakaźnej. U 40 (24,09%) dzieci spośród 166 pacjentów zakwalifikowanych do badania po uwzględnieniu warunków wyłączenia nie występowały klasyczne objawy mononukleozy zakaźnej (gorączka, powiększenie węzłów chłonnych, zapalenie gardła i migdałków podniebiennych). Chłopcy stanowili 40,96% (68 z 166) badanej grupy. Dominowały dwie grupy wiekowe: 4 – 10 lat (27,71%) i 14 – 18 lat (48,19%). Objawy trwały średnio 14,4 ± 5,3 dnia, a hospitalizacja: 4,7 ± 2,2 dnia. Częstość występowania objawów była następująca: limfadenopatia – 92,77%, zapalenie gardła – 90,36%, gorączka – 86,14%, splenomegalia – 66,87%.

Częstość występowania objawów świadczących o patologii wątroby i dróg żółciowych wynosiła: hepatomegalia – 80,72%, ból brzucha – 31,32%, nudności/ wymioty – 27,11%, żółtaczka – 11,44%, świąd skóry – 11,44%, tkliwość palpacyjna brzucha – 10,84%, ściemnienie moczu – 4,82%, odbarwienie stolca – 1,20%. U trójki pacjentów (1,81%) rozpoznano sepsę. Powikłania pozawątrobowe rozwinęło 48,19% dzieci, najczęściej obserwowano obturację dróg oddechowych (41,56%).

Podwyższoną aktywność aminotransferazy alaninowej (ALT) i asparaginianowej (AST) obserwowano od pierwszego do trzeciego tygodnia choroby z tendencją do normalizacji od czwartego tygodnia. Aktywność ALT przekraczała pięciokrotność wartości górnej granicy normy dla płci i wieku najczęściej w pierwszym tygodniu choroby u 44/95 (46,31%) pacjentów. Aktywność AST przekraczała pięciokrotność wartości górnej granicy normy najczęściej w drugim tygodniu choroby u 33/108 (30,55%) dzieci. Aktywność ALT utrzymywała się powyżej normy przez 3 tygodnie, zaś aktywność AST wykazywała dwa szczyty: w pierwszym tygodniu (średnio 186,34 ± 148,17 IU/l) oraz w trzecim tygodniu (średnio 169,00 ± 140,98 IU/l). Zmiany średniej aktywności ALT i AST w kolejnych tygodniach choroby przedstawia rycina 1.



Ryc. 1. Dynamika zmian średniej aktywności ALT i AST w kolejnych tygodniach obserwacji.
Skróty: ALT – aminotransferaza alaninowa, AST – aminotransferaza asparaginianowa.

Aktywność GGTP była podwyższona w pierwszych trzech tygodniach choroby osiągając najwyższe wartości w drugim tygodniu (średnio 183,34 ± 97,48 IU/l). U 60,53% pacjentów występowało podwyższone stężenie bilirubiny całkowitej. Stężenie bilirubiny związanej przekraczało 20% stężenia bilirubiny całkowitej u 18 (47,37%) dzieci, w każdym przypadku wiązało się to z podwyższoną aktywnością GGTP.

U 18/166 (10,84%) pacjentów z zapaleniem wątroby obserwowano kliniczne i laboratoryjne objawy cholestazy, a średni wiek tych pacjentów wynosił 13,00 ± 5,23 lat. Dwanaścioro (12/18, 66,66%) z nich było starszych niż 15 lat. U wszystkich pacjentów z objawami cholestazy występowały ultrasonograficzne cechy bezkamiczego zapalenia pęcherzyka żółciowego (pogrubienie ściany pęcherzyka żółciowego powyżej 3 mm, obecność płynu wokół pęcherzyka).

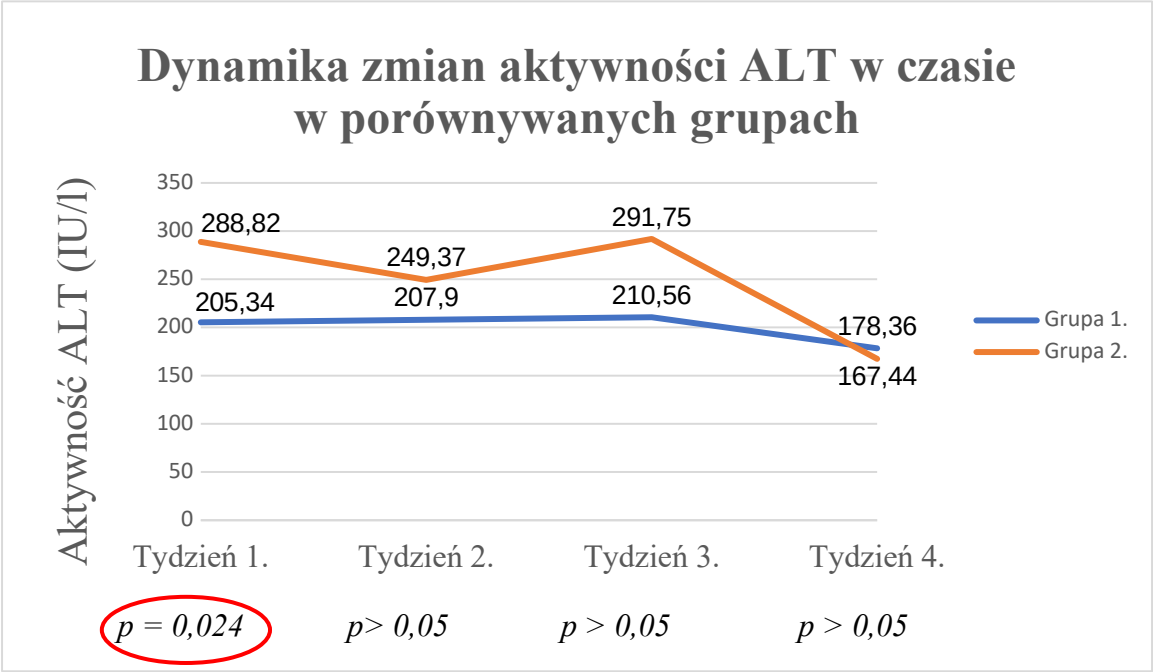
Nieprawidłowości w badaniu USG jamy brzusznej wykazano u 95,68% (11 z 116) dzieci, występowały one z następującą częstością: splenomegalia – 85,34%, hepatomegalia – 62,07%, limfadenopatia wnęki wątroby – 55,17%, pogrubienie ściany pęcherzyka żółciowego – 14,65%.

W **Publikacji nr 2.** zidentyfikowano istotne statystycznie różnice pomiędzy badanymi grupami w przebiegu klinicznym zapalenia wątroby pierwotnej infekcji EBV, które dotyczyły:

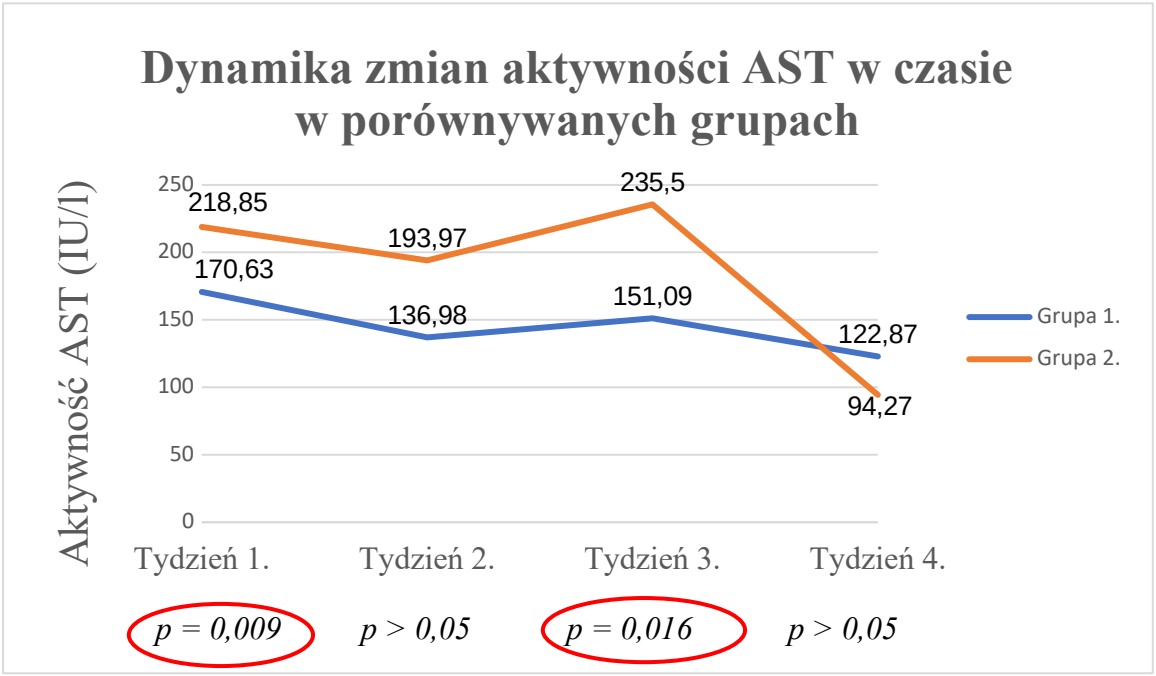
1. czasu trwania hospitalizacji – była ona dłuższa w grupie pacjentów leczonych glikokortykosteroidami: 5,0 ± 2,1 dni vs. 4,3 ± 2,3 dni dla grupy drugiej (dzieci nieotrzymujące glikokortykosteroidów), $p = 0,015$;
2. częstości występowania klasycznych objawów mononukleozy zakaźnej (gorączka, limfadenopatia, zapalenie gardła i migdałków podniebiennych) – obserwowano je częściej u pacjentów leczonych glikokortykosteroidami: 84,61% vs. 61,29% w grupie 2, $p = 0,000$;
3. występowania gorączki, którą częściej obserwowano u pacjentów leczonych glikokortykosteroidami: 90,38% vs. 79,03% w grupie drugiej, $p = 0,040$;
4. czasu trwania gorączki, która utrzymywała się dłużej u pacjentów zakwalifikowanych do leczenia glikokortykosteroidami: 7,5 ± 4,7 dni vs. 5,9 ± 4,8 dni u dzieci nieotrzymujących glikokortykosteroidów, $p = 0,049$;
5. częstości występowania powiększenia wątroby w badaniu palpacyjnym brzucha, co stwierdzano u 85,58% dzieci z grupy pierwszej vs. 72,58% z grupy drugiej, $p = 0,040$; podobnie powiększenie śledziony częściej stwierdzano u dzieci otrzymujących glikokortykostaroidy: 73,08% vs. 56,45% w grupie 2, $p = 0,027$.

Istotne różnice w zakresie wyników badań laboratoryjnych dotyczyły:

1. odsetka neutrofilów w rozmazie ręcznym krwinek białych, który był istotnie wyższy u pacjentów wymagających steroidoterapii: 33,5% ± 12,2% vs. 19,4% ± 14,5% w grupie 2, $p = 0{,}020$;
2. stężenia CRP (*C-reactive protein*), które było wyższe u pacjentów z pierwszej grupy: 25,51 mg/dl ± 17,92 mg/dl vs. 15,46 mg/dl ± 11,11 mg/dl w grupie 2, $p = 0{,}000$;
3. średniej aktywności ALT w pierwszym tygodniu choroby, która była istotnie wyższa u dzieci nieotrzymujących glikokortykosteroidów: 288,82 ± 170,16 IU/l (50,00 – 725,00) vs. 205,34 ± 115,40 IU/l (39,00 – 516,00) w grupie pierwszej, $p = 0{,}024$; różnice obserwowane w kolejnych tygodniach badania nie były istotne;
4. średniej aktywności AST, która była w obu grupach najwyższa w pierwszym i trzecim tygodniu choroby, jednak różnice te były istotne statystycznie jedynie w grupie dzieci nieleczonych glikokortykosteroidami ($p = 0{,}024$); średnia aktywność AST istotnie różniła się pomiędzy grupami w pierwszym i trzecim tygodniu choroby: w 1. tygodniu wynosiła 170,63 ± 159,47 IU/l (40,00 – 852,00) vs. 218,85 ± 128,22 IU/l (42,00 – 589,00) odpowiednio w grupie 1. i 2., ($p = 0{,}009$); zaś w 3. tygodniu choroby wynosiła 151,09 ± 138,57 IU/l (31,00 – 578,00) vs. 235,50 ± 170.27 IU/l (67,00 – 617,00) odpowiednio w grupie 1. i 2., ($p = 0{,}016$). Dynamikę zmian średniej aktywności ALT i AST w kolejnych tygodniach choroby w porównywanych grupach pacjentów przedstawiono na rycinach 2. i 3.



Ryc. 2. Dynamika zmian średniej aktywności ALT w kolejnych tygodniach choroby w porównywanych grupach pacjentów. Grupa 1 – pacjenci leczeni glikokortykosteroidami z powodu powikłań mononukleozy zakaźnej. Grupa 2 – pacjenci nieotrzymujący glikokortykosteroidów. Skróty: ALT – aminotransferaza alaninowa.



Ryc. 3. Dynamika zmian średniej aktywności AST w kolejnych tygodniach choroby w porównywanych grupach pacjentów. Grupa 1 – pacjenci leczeni glikokortykosteroidami z powodu powikłań mononukleozy zakaźnej. Grupa 2 – pacjenci nieotrzymujący glikokortykosteroidów. Skróty: AST – aminotransferaza asparaginianowa.

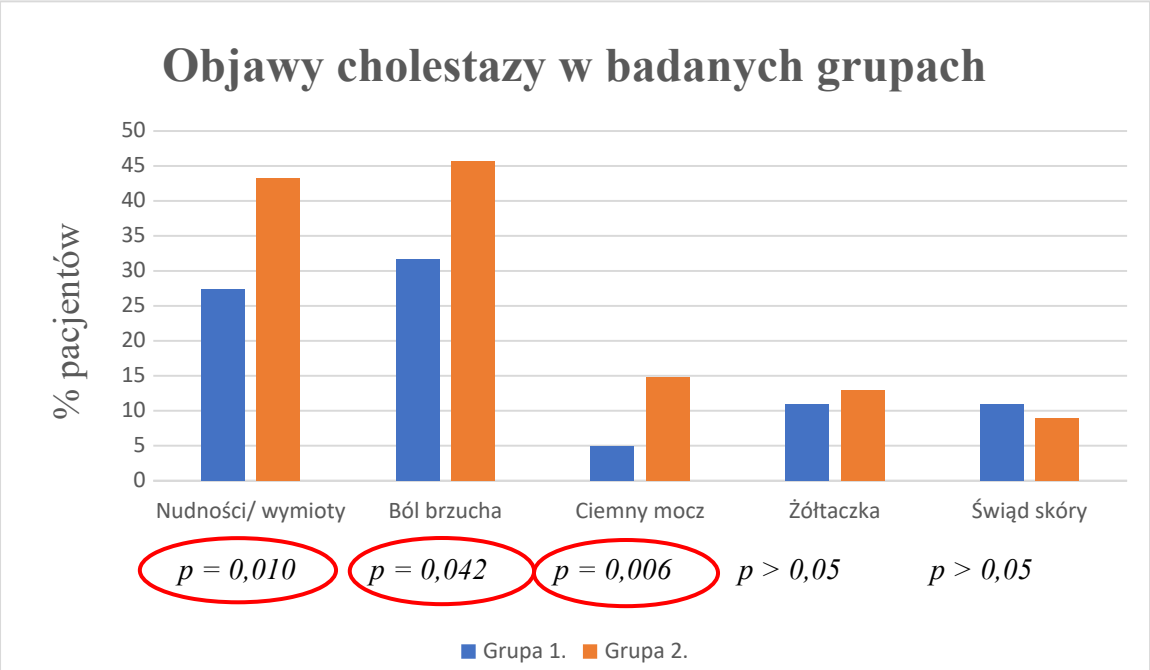
Należy podkreślić, że nie stwierdzono istotnej różnicy w częstości występowania nieprawidłowości w badaniu USG jamy brzusznej (w tym także hepatomegalii i splenomegalii) pomiędzy badanymi grupami.

Pacjenci z pierwszej grupy istotnie częściej byli leczeni antybiotykiem (najczęściej z powodu podejrzenia bakteryjnej infekcji gardła) przed przyjęciem do szpitala – 68 (65,40%) w porównaniu z grupą 2. – 23 (37,10%), $p = 0,000$.

W **Publikacji nr 3.** wykazano statystycznie istotne różnice w przebiegu zapalenia wątroby pomiędzy dziećmi z potwierdzoną serologicznie pierwotną infekcją EBV (grupa 1.) a pacjentami z serologicznymi cechami pierwotnej koinfekcji EBV i CMV (grupa 2.). Dotyczyły one częstości występowania poszczególnych objawów choroby:

1. zapalenie gardła i migdałków podniebiennych częściej obserwowano w pierwszej grupie - u 148 (90,24%) vs. 65 (80,25%) w grupie drugiej, $p = 0,047$;
2. wymioty/ nudności częściej występowały w grupie 2. – 35 (43,21%) vs. 45 (27,44%) w grupie 1., $p = 0,010$ (OR = 2,01 [95% CI: 1,15 – 3,51]);
3. ból brzucha zgłaszało 37 (45,67%) dzieci z drugiej grupy vs. 52 (31,71%) dzieci z pierwszej grupy, $p = 0,042$ (OR = 1,81 [95% CI: 1,05 – 3,13]);
4. ściemnienie moczu obserwowano częściej u pacjentów z grupy 2. – 12 (14,81%) niż w grupie 1. – 8 (4,88%), $p = 0,006$ (OR = 3,39 [95% CI: 1,33 – 8,67]).

Częstość występowania objawów cholestazy w badanych grupach przedstawiono na rycinie 4.



Ryc. 4. Częstość występowania poszczególnych objawów cholestazy w badanych grupach. Grupa 1 – pacjenci z zapaleniem wątroby w przebiegu pierwotnego zakażenia EBV. Grupa 2 – pacjenci z zapaleniem wątroby w przebiegu koinfekcji EBV/ CMV potwierdzonej serologicznie.

W zakresie diagnostyki laboratoryjnej i obrazowej istotne różnice dotyczyły:

1. stężenia CRP, które było wyższe u pacjentów z pierwszej grupy – 21,93 ± 16,54 mg/dl niż w grupie drugiej – 15,15 ± 12,85 mg/dl, $p = 0{,}001$;
2. odsetka granulocytów obojętnochłonnych w rozmazie ręcznym krwinek białych, który był wyższy w pierwszej grupie – 32,07 ± 13,25% niż w drugiej grupie – 25,33% ± 12,35%, $p = 0{,}000$;
3. częstości uzyskiwania nieprawidłowych wyników badania USG jamy brzusznej, co częściej zdarzało się w grupie 2. (u 100% badanych) niż w grupie 1. (66,46%), $p = 0{,}045$; poszczególne nieprawidłowości (hepatomegalia, powiększenie węzłów chłonnych wnęki wątroby, patologie pęcherzyka żółciowego i dróg żółciowych) uwidaczniano częściej w drugiej grupie, jednak różnice pomiędzy grupami nie były istotne.

Kryteria cięższego przebiegu choroby (stworzone na potrzeby niniejszego badania) istotnie częściej spełniały dzieci z grupy 2. – 60 (74,07%) vs. 96 (58,54%) w grupie 1., $p = 0{,}018$ (OR = 2,02 [95% CI: 1,13 – 3,64]).

Dzieci z grupy pierwszej istotnie częściej wymagały antybiotykoterapii po przyjęciu do szpitala: 83 (50,61%) niż dzieci z grupy drugiej: 27 (33,33%), $p = 0{,}011$.

Wnioski

1. U dzieci z mononukleozą zakaźną ocena aktywności ALT, AST, GGTP oraz USG jamy brzusznej mają istotne znaczenie w kontekście zidentyfikowania pacjentów z cięższym, cholestatycznym przebiegiem zapalenia wątroby lub bezkamiczym zapaleniem pęcherzyka żółciowego, którzy wymagają wnikliwej kontroli po ustąpieniu ostrych objawów choroby (**Publikacja nr 1.**).
2. Steroidoterapia (najczęściej stosowana celem leczenia obturacji dróg oddechowych w przebiegu mononukleozy zakaźnej) może łagodzić przebieg zapalenia wątroby w pierwotnej infekcji EBV u dzieci (zwłaszcza w pierwszym tygodniu choroby), jednak nie wpływa na wartości parametrów cholestazy i częstość występowania nieprawidłowości dotyczących wątroby i dróg żółciowych uwidacznianych w USG (nie modyfikuje ryzyka rozwoju powikłań). Wczesne obniżenie aktywności ALT i AST u dzieci leczonych steroidami może maskować rzeczywistą dynamikę choroby, dlatego kluczowa jest kontrola aktywności tych enzymów po zakończeniu steroidoterapii (**Publikacja nr 2.**).
3. U dzieci z zapaleniem wątroby w przebiegu pierwotnego zakażenia EBV pozytywny wynik oznaczenia przeciwciał IgM dla CMV wiązał się z większym nasileniem objawów cholestazy i częstszym występowaniem nieprawidłowości dotyczących wątroby i dróg żółciowych w USG. U tych pacjentów obserwowano też cięższy przebieg zapalenia wątroby (wyższa aktywność ALT i AST) w początkowej fazie choroby (**Publikacja nr 3.**).

Oświadczenia wszystkich współautorów publikacji określające indywidualny wkład (udział merytoryczny i procentowy) każdego z nich w ich powstanie.

Warszawa, 30.12.2025
(miejscowość, data)

Maria Pokorska-Śpiewak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Epstein Barr Virus Hepatitis – A Mild Clinical Symptom or a Threat?" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 20%.

Wkład Magdaleny Rutkowskiej w powstawanie publikacji określam jako 80%,

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obejmował on: koncepcję pracy, metodykę, zbieranie danych, analizę danych, interpretację wyników, napisanie manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Rutkowskiej.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 30.12.2025
(miejscowość, data)

Maria Pokorska-Śpiewak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The influence of steroid therapy of complications of infectious mononucleosis on the course of Epstein-Barr virus hepatitis." oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%.

Wkład Magdaleny Rutkowskiej w powstawanie publikacji określam jako 90%,

(imię i nazwisko kandydata do stopnia)

obejmował on: koncepcję pracy, metodykę, zbieranie danych, analizę danych, interpretację wyników, napisanie manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Rutkowskiej.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 30.12.2025
(miejscowość, data)

Maria Pokorska-Śpiewak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „The impact of acute CMV coinfection on the course of EBV hepatitis in children." oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: 10%.

Wkład Magdaleny Rutkowskiej w powstawanie publikacji określam jako 90%,

(imię i nazwisko kandydata do stopnia)

obejmował on: koncepcję pracy, metodykę, zbieranie danych, analizę danych, interpretację wyników, napisanie manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Magdaleny Rutkowskiej.

(imię i nazwisko kandydata do stopnia)

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

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