Features and prognosis of cytomegalovirus-associated biliary atresia: a retrospective study

Zhe Wang
Guangzhou Women and children's Medical Center
Xi-Si Guan
Guangzhou Women and Children's Medical Center
Yue Wu
Guangzhou Women and Children's Medical Center
Qiu-Ming He
Guangzhou Women and Children's Medical Center
Le Li
Guangzhou Women and Children's Medical Center
Jia-Kang Yu
Guangzhou Women and Children's Medical Center
Wei Zhong (zhongwei@gwcmc.org)
Guangzhou Women and Children's Medical Center https://orcid.org/0000-0002-7428-9834

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Abstract

Purpose

To investigate the relationship between cytomegalovirus (CMV) infection and biliary atresia (BA) onset, development and short-term prognosis after Kasai operation.

Methods

A retrospective study was conducted. BA Patients with obstructive jaundice and tested for CMV infection were included and grouped by CMV-IgM and CMV-DNA test results, between-group differences of preoperative blood tests and short-term prognosis indicators were investigated for the statistical significance.

Results

the CMV infection rate was higher in BA patients compared with non-BA jaundiced patients. Higher preoperative gamma-glutamyl transferase (GGT) level and lymphocyte percentage (Lym%) were significantly corelated with the CMV infection in BA patients. CMV(+) BA Patients had similar short-term outcome comparing with CMV(-) patients. IgM(+)DNA(+) group had highest GGT, total bilirubin (TBiL) and direct bilirubin (DBiL) level. IgM(-)DNA(+) group had the lowest GGT and the highest alkaline phosphatase (ALP) level. IgM(+)DNA(-) group had the highest bodyweight and lymphocyte percentage. The IgM (+)DNA (-) group had more patient achieved complete jaundice clearance than other groups.

Conclusion

CMV infection may associate to BA development and progression. Perioperative antivirus treatments may be necessary for improving outcome. Better elucidation of the underlying mechanism will require further investigation.

Introduction

Biliary atresia (BA) is the major cause of infantile obstructive jaundice, manifested as rapid progression of intrahepatic and extrahepatic bile duct fibrosis. Even when adequate surgical treatment is performed, a significant proportion of patients still need liver transplantation in their life[1].

The cause of BA is still unclear. The theory of viral infection has been extensively explored in recent studies[2]. Cytomegalovirus (CMV) infection may lead to a variety of neonatal diseases including obstructive jaundice and CMV hepatitis[3]. Previous studies have identified a correlation between CMV infection and BA[4]. Therefore, some scholars have suggested that BA-associated CMV infection (CMV-

BA) may be a subgroup of BA with its own unique clinical manifestations[5,6]. However, these have been relatively small-scale studies. The clinical features of CMV-positive BA have not been described in a large case series. The current study is a large retrospective review focusing on illustrating the feature of CMV-BA and elucidating the intrinsic link between CMV infection and BA development.

Materials And Methods

Patients with obstructive jaundice, investigated for both CMV-IgM and CMV-DNA were included, and electronic medical records were extracted and retrospectively reviewed in the pediatric surgery department of Guangzhou Woman and Children's Medical Center. Patients' general information, definitive diagnosis, preoperative liver function, blood count, preoperative CMV test results, age at Kasai, total bilirubin (TBIL) values at the first, third and the latest months post-surgery were collected.

The Jaundice clearance (JC) was defined as TBIL≤25 μmol/L. Complete JC was defined as the achievement of JC during the period from Kasai to last follow-up. Early JC was defined as achievement of JC within 1 month after Kasai. Persistent JC was defined as TBIL remaining less than 25μmol/L until last follow-up.

The CMV-IgM level in this study was determined via enzyme-linked immunosorbent assay and the results were indicated as positive or negative. The CMV-DNA was tested by PCR-fluorescence, results were indicated as positive if the copy number was equal to or greater than 500 cp/ml, or negative if the copy number was less than 500 cp/ml. None of the patients received CMV anti-viral treatments before surgical intervention.

Data were presented as mean±standard error of mean (SEM), or percentage. Categorical data were analyzed by chi-square test or Fisher exact test, continuous data were analyzed by unpaired t-test or nonparametric test (Mann-Whitney test). Dunn's multiple comparisons test was used to compare more than two groups' nonparametric data. The above analysis was performed with GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego California USA). One-way correlation analysis and Multiple regression was performed with Empower(R) (www.empowerstats.com, X&Y solutions, Inc. Boston MA) and R (http://www.R-project.org).

Results

A total of 557 obstructive jaundice patients were investigated for CMV infection (IgM or DNA) during December 30, 2008 to December 31, 2018. Patients with incomplete test results (Only IgM or DNA result) were excluded, 347 patients with both IgM and DNA results were included. Of those, 32 of them were non-BA jaundice patients and 315 were diagnosed as BA by cholangiography. Among the 315 BA patients, 129 underwent Kasai operation, 186 patients only had cholangiography because of delayed diagnosis (the parents chose liver transplantation instead of Kasai) or cultural economic reasons. Preoperative liver

function was reviewed in 193 patients. Of the 129 patients who underwent Kasai operation, 91 had short-term prognostic data *(Figure 1).*

1. CMV Infection Rate

Either CMV-IgM or CMV-DNA was positive in 150 patients who were final diagnosed as BA by cholangiography, these patients were considered CMV positive. CMV-IgM and CMV-DNA were both negative in 165 BA patients, they were considered CMV negative. In 32 non-BA patients, 11 of them were CMV(+), The CMV infection rate in BA patients was higher than that in non-BA obstructive jaundice patients (150/315, 47.6% vs 11/32, 34.4%), however, the difference was not significant (p = 0.15) (*Table 1).*.

2. CMV-IgM, CMV-DNA and Preoperative Parameters of BA patients

BA Patients positive for CMV infection had significantly greater preoperative gamma-glutamyl transpeptidase (GGT), total bilirubin (TBiL) and direct bilirubin (DBiL) level than CMV negative BA patients (736.8±72.2 U/L vs 568.0±33.5 U/L, p = 0.02, 165.8±5.7 μmol/L vs 155.9±5.7 μmol/L, p = 0.04 and 136.4±4.8 μmol/L vs 126.6±4.0 μmol/L, p = 0.05, respectively). The mean preoperative bodyweight of the CMV(+) group was 5.1±0.1 kg, significantly heavier than the CMV(-) group (4.6±0.1 kg, P = 0.0001). The age at Kasai also older in the CMV(+) group than in the CMV(-) group (81.5±3.4 days vs 71.6±2.3 days, p = 0.01). The preoperative lymphocyte percentage (Lym%) was significantly higher in the CMV(+) patients than in the CMV(-) patients (62.4±1.2 percent vs 58.5±1.0 percent, p = 0.004).

Between DNA(+) and DNA(-) patients, the TBiL and DBiL level were higher in the DNA(+) patients than in the DNA(-) patients (174.0±7.0 μmol/L vs 154.9±4.9 μmol/L and 143.4±5.6 μmol/L vs 126.0±3.6 μmol/L), the difference was statistically significant (p = 0.003 and p = 0.002, respectively). Bodyweight of DNA(+) patients were significantly greater than DNA(−) patients (5.1±0.8 kg vs 4.7±0.9 kg, p = 0.02). The age at Kasai was not significantly different between these two groups (80.3±3.8 days vs 73.8±2.3 days).

Between IgM(+) and IgM(-) patients, the GGT level was significantly higher in the IgM(+) patients than in the IgM(-) patients (898.0±92.9 U/L vs 533.3±29.7 U/L, p<0.0001). The preoperative Lym% was significantly higher in the IgM(+) patients than in the IgM(-) patients (62.6±1.5 percent vs 59.1±0.9 percent, p = 0.02). The age at Kasai and the preoperative bodyweight were greater in IgM(+) group than in IgM(-) group (82.6±4.1 days vs 72.8±2.2 days, p = 0.01 and 5.1±0.1/ kg vs 4.7±0.1 kg, p = 0.001, respectively). *(Table 2)*

The age may act as an independent influencing factors and be responsible for the elevation of preoperative liver function levels. Therefore, we performed one-way correlation analysis on the effects of CMV(+)/(-), IgM(+)/(-), DNA(+)/(-), and age at Kasai on preoperative live function parameters. We found that the older age not only related to the elevation of GGT level (OR = 2.5, 95%CI = 0.0 to 5.0, p = 0.05), but

also affect the ALT (OR = 0.9, 95%CI = 0.2 to 1.5, p = 0.01), DBiL (OR = 0.3, 95%CI = 0.1 to 0.5, p = 0.01) and TBA level (OR = 0.9, 95%CI = 0.5 to 1.2, p<0.0001) *(Table 3).*

Multiple regression was performed to adjust the confounding effect of age at Kasai and estimate the independent relationship between preoperative liver function and CMV infection *(Table 4).* CMV(+) was associated with elevation of GGT and Lym% (adjusted β = 148.6, 95%CI = 6.3 to 290.8, p = 0.04, and adjusted β = 4.4, 95%CI = 1.3 to 7.5, p = 0.006, respectively). CMV IgM(+) was also associated with high GGT and Lym% (adjusted β = 349.2, 95%CI = 200.9 to 497.6, p<0.0001, and adjusted β = 3.8, 95%CI = 0.4 to 7.2, p = 0.03, respectively), CMV DNA(+) only associated with high DBiL level (adjusted β = 15.6, 95%CI = 2.2 to 29.0, p<0.02).

Then the BA patients were divided into four subgroups according to different combination of CM IgM and CMV DNA test results, they were IgM(+)DNA(+), IgM(+)DNA(-), IgM(-)DNA(+) and IgM(-)DNA(-) group. IgM(+)DNA(+) group had highest GGT level but the lowest alkaline phosphatase (ALP) level, on contrary, IgM(-)DNA(+) group had the lowest GGT and the highest ALP level. IgM(+)DNA(-) group had the highest bodyweight. The age at Kasai had no statistical differences between all groups *(Figure 2).*

3. CMV-IgM, CMV-DNA and short-term prognosis parameters after Kasai

There were more patients with a better prognosis in the CMV(-) group than in the CMV(+) group (complete JC 50.0% vs 48.0%, persistent JC 40.0% vs 32.0%), but the difference was not significant *(Table 5).* The IgM(+)DNA(−) subgroup had significantly more patients achieved complete JC comparing with other groups. *(Table 6).*

Discussion

Great efforts to reveal the etiology of BA have been made in recent years[7], but much remains unknown. We know little about the time of onset of the disease, and it appears that the pattern and speed of disease progression vary among patients; although surgical intervention occurs at a significantly younger age, all patients scheduled for the procedure already have some degree of liver damage[8]. These issues make it difficult to diagnose and treat BA.

The prevalence of BA in the Chinese population is higher comparing to the Caucasian population[9]. At the same time, the rate of cytomegalovirus infection in Chinese population is also higher than in western countries. Is there an intrinsic link between the two phenomena? Viral pathogenesis is one important hypothesis of BA etiology[1]. Prenatal CMV infection causes liver and biliary system damage, presenting as viral hepatitis and obstructive jaundice[3]. Thus, CMV is considered an important causative agent in BA. Consistent with the higher incidence of BA, the prevalence rate of CMV in pregnant women is higher in China than in Western countries[10, 11]. All these findings support the CMV hypothesis. However, the clinical presentation and pathological change of CMV hepatitis is different from BA[3] and there is no

direct evidence to suggest that CMV hepatitis can develop into the latter. But recent studies do show a possible association between CMV infection and onset of BA[4, 5, 12, 13]. The role of CMV in the pathogenesis of BA still lacks in-depth research.

In this study, we retrospectively reviewed 347 obstructive jaundice patients invested for CMV infection. Compared with the 0.5–0.7% CMV infection rate in the Chinese newborn population, the CMV-positive rate of non-BA obstructive jaundice patient population was 34.4% and BA patients was 47.6%; both rates are significantly higher than the background prevalence incidence and are even higher in BA patients. These results are in accordance with previous studies[4, 13].

According to our results, CMV(+) patients were significantly older than CMV(-) patients when they receive Kasai operation. Caponcelli E et al also found that the CMV positive patients were older in their case serie[6]. CMV infection rate increaces with age, this might be the cause of older positive patients. After adjusted with age, CMV infection still had strong association with the elevation of GGT and Lym%. Since more GGT in serium indicates more severe the biliary epithelial injury, it is reasonable to suspect that, independent of disease progression, CMV infection correlated with the damaging of the biliary system in BA patients.

The timeframe for detection of CMV-IgM and CMV-DNA in the peripheral blood after the initial infection is known to be not completely synchronized[14, 15]. A previous study showed that CMV-IgM can be detected in serum 3 to 4 weeks after the primary infection and decreases to undetectable levels by 3 to 4 months after infection[14]. The different positive combination of IgM and DNA may provide some clue about the different timing of CMV infection. Although the time periods may overlap and there may be individual differences, we believe the IgM(+)DNA(+) patients might get the infection earlier than the IgM(-)DNA(+) patients, because IgM(-)DNA(+) patients' bodies were not producing IgM yet, and the IgM(+)DNA(-) patients may be infected at an earliest time because the virus had been cleared out of their bodies when we ran the tests. The GGT level exhibited a pattern of raise and decline among different IgM and DNA combination groups: it started at a low level in IgM(-)DNA(+) patients, reached the peak in IgM(+)DNA(+) and declined in IgM(+)DNA(-), further proofed that the CMV infection related to the preoperative GGT raising in the BA patients. This hypothesis is also supported by the increasing lymphocyte's percentage in BA patients in this study. We speculate that CMV may not only be a "passenger infection" but might also associate with the disease progression.

The CMV(-) patients had better prognosis comparing with CMV(+) patients, this is consistent with the result of previous study[6]. But unlike the strong association between CMV infection and worse outcome as previously reported, our result was not statistically significant. We think this inconsistency may cause by the relatively small CMV positive prognosis group in both researches (20 in report vs 40 in present study), also the time of the CMV infection, individual immune difference and the characteristic of BA itself can affect the outcome, further study should include more case to eliminate variation.

The limitation of this study was incompleteness of data. Patients transferred from the Pediatric Infectious and Liver Diseases Department were investigated for CMV infection routinely while patients

admitted directly in Pediatric Surgery Department were not, this difference might cause selection bias and weak the result. Although the study population was large, some patients did not have data available for further analysis, weakening the statistic power of the result. The missing data led to some study results that were obscure and difficult to interpret.

Conclusion

CMV infection in early life is related to elevated preoperative levels of GGT and Lym% in patients with BA. CMV infection may associate to BA development and progression. Better elucidation of the underlying mechanism will require further investigation.

Declarations

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Compliance with ethical standards

Conflict of interest:

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Ethical approval

This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The medical information used in this study was approved by patients' parents and written conceit was signed. The study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center.

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Tables

Table 1. CMV-IgM and CMV-DNA results in different diagnosis groups

	CMV-IgM (+/-)	CMV-DNA (+/-)	CMV(+)/CMV(-)	CMV positive rate
BA (n=315)	102/213	96/219	150/165	47.6%
Non-BA (n=32)	6/26	10/26	11/21	34.4%

* CMV(-)=CMV-IgM(-) AND CMV-DNA(-), CMV(+)=CMV-IgM(+) OR CMV-DNA(+), BA = biliary atresia, CMV = cytomegalovirus.

Table 2. Comparison between CMV(+)/CMV(-), CMV-DNA(+)/DNA(-) and CMV-IgM(+)/IgM(−) BA patients in preoperative parameters

	CMV(+)/CMV(-)	DNA(+)/DNA(-)	IgM(+)/IgM(-)
	77/116	50/143	54/139
ALT	184.8±16.4/172.8±11.2	197.1±23.3/170.8±9.7	181.7±19.8/176.0±10.5
AST	253.1±24.72/240.0±14.4	273.7±36.7/235.3±12.2	247.8±33.4/244.3±12.9
GGT	736.8±72.2/568.0±33.5[a]	685.4±88.6/617.8±36.7	898.0±92.9/533.3±29.7[b]
TBiL	165.8±5.7/155.9±5.7[c]	174.0±7.0/154.9±4.9[d]	164.1±7.4/158.3±4.9
DBiL	136.4±4.8/126.6±4.0[e]	143.4±5.6/126.0±3.6[f]	135.2±6.2/128.7±3.5
TBA	169.6±9.0/162.1±6.9	177.1±12.2/160.9±6.0	165.8±10.4/164.8±6.4
ALP	513.8±24.4/480.3±18.8	521.0±31.4/484.1±16.9	470.5±26.7/503.5±17.8
Weight	5.1±0.1/4.6±0.1[g]	5.1±0.8/4.7±0.9[h]	5.1±0.1/4.7±0.1[i]
Age at Kasai	81.5±3.4/71.6±2.3[j]	80.3±3.8/73.8±2.3	82.6±4.1/72.8±2.2[k]
Lym%	62.4±1.2/58.5±1.0[l]	62.3±1.4/59.3±0.9	62.6±1.5/59.1±0.9[m]
Lym	7.5±0.3/7.0±0.2	7.4±0.3/7.1±0.2	7.4±0.3/7.1±0.2

p_a=0.02, p_b=<0.0001, p_c=0.04, p_d=0.003, p_e=0.05, p_f=0.002, p_g=0.0001, p_h=0.02, p_i=0.001, p_j=0.01, p_k=0.01, p_l=0.004, p_m=0.02

CMV = cytomegalovirus, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, TBIL = total bilirubin, DBIL = direct bilirubin, TBA = total bile acids, ALP = alkaline phosphatase, Lym% = percentage of lymphocytes, Lym= lymphocytes count.

Table 3. One-way correlation analysis between preoperative parameters and CMV(+)/CMV(-), DNA(+)/DNA(-), IgM(+)/IgM(-) and age at Kasai.

	CMV(+)/CMV(-)	DNA(+)/DNA(-)	IgM(+)/IgM(-)	Age at Kasai
ALT	12.0 (-25.6, 49.5)	26.4 (-15.5, 68.2)	5.7 (-35.3, 46.6)	**0.9 (0.2, 1.5)[a]**
AST	13.1 (-39.5, 65.6)	38.3 (-20.2, 96.9)	3.6 (-53.8, 60.9)	0.6 (-0.3, 1.5)
GGT	**168.8 (28.2, 309.3)[b]**	67.6 (-91.5, 226.6)	**364.8 (218.1, 511.4)[b]**	**2.5 (-0.0, 5.0)[d]**
TBiL	9.9 (-6.5, 26.3)	**19.1 (0.9, 37.3)[e]**	5.8 (-12.1, 23.8)	0.3 (-0.0, 0.6)
DBiL	9.9 (-2.4, 22.1)	**17.4 (3.8, 30.9)[f]**	6.5 (-6.8, 19.9)	**0.3 (0.1, 0.5)[g]**
TBA	7.5 (-14.4, 29.4)	16.2 (-8.1, 40.6)	1.1 (-22.8, 25.0)	**0.9 (0.5, 1.2)[h]**
ALP	32.5 (-27.0, 91.9)	36.1 (-30.4, 102.5)	-32.4 (-97.4, 32.7)	0.2 (-0.9, 1.3)
Lym%	**3.9 (0.8, 7.0)[i]**	3.0 (-0.5, 6.5)	**3.4 (-0.0, 6.8)[j]**	-0.0 (-0.1, 0.0)
Lym	0.6 (-0.2, 1.3)	0.2 (-0.6, 1.0)	0.3 (-0.5, 1.0)	0.0 (-0.0, 0.0)

OR(95%CI), p_a=0.01, p_b=0.02, p_c=<0.0001, p_d=*0.05,* p_e=0.04, p_f=0.01, p_g=0.01, p_h=<0.0001, p_i=0.01, p_j=0.05

ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, TBIL = total bilirubin, DBIL = direct bilirubin, TBA = total bile acids, ALP = alkaline phosphatase, Lymph% = percentage of lymphocytes.

Table 4. Multiple regression analysis between preoperative parameters and CMV(+)/CMV(-), DNA(+)/DNA(-) and IgM(+)/IgM(-), adjusted by age at Kasai

	CMV(+)/CMV(-)	DNA(+)/DNA(-)	IgM (+)/IgM(-)
ALT	3.3 (-34.3, 40.9)	20.9 (-20.6, 62.4)	-3.1 (-43.9, 37.8)
AST	7.2 (-46.2, 60.5)	34.7 (-24.1, 93.5)	-2.5 (-60.6, 55.5)
GGT	**148.6 (6.3, 290.8)[a]**	51.9 (-107.0, 210.8)	**349.2 (200.9, 497.6)[b]**
TBiL	7.4 (-9.2, 24.0)	17.5 (-0.7, 35.7)	3.2 (-14.9, 21.3)
DBiL	7.2 (-5.1, 19.4)	**15.6 (2.2, 29.0)[c]**	3.8 (-9.6, 17.1)
TBA	-1.2 (-22.4, 20.0)	10.7 (-12.6, 34.1)	-7.6 (-30.6, 15.4)
ALP	31.6 (-28.9, 92.1)	35.2 (-31.7, 102.2)	-35.2 (-101.2, 30.8)
Lym%	**4.4 (1.3, 7.5)[d]**	3.3 (-0.2, 6.8)	**3.8 (0.4, 7.2)[e]**
Lym	0.5 (-0.3, 1.2)	0.1 (-0.7, 0.9)	0.2 (-0.6, 1.0)

Adjusted β (95%CI), p_a=0.04, p_b=0.0001, p_c=0.02, p_d=0.006, p_e=0.03

ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, TBIL = total bilirubin, DBIL = direct bilirubin, TBA = total bile acids, ALP = alkaline phosphatase, Lymph% =

percentage of lymphocytes.

Table 5. Prognosis factors comparison between CMV(+)/CMV(-), DNA(+)/DNA(-), and IgM(+)/IgM(-) groups.

	CMV(+)/CMV(-)	DNA(+)/DNA(-)	IgM(+)/IgM(-)
	40/50	30/60	25/65
Complete JC (n=44)	50.0%/48.0% (20/24)	40.0%/53.3% (12/32)	52.0%/47.7% (13/31)
Early JC (n=11)	10.0%/14.0% (4/7)	3.3%/16.7% (1/10)	16.0%/10.8% (4/7)
Persistent JC (n=32)	40.0%/32.0% (16/16)	33.3%/36.7% (10/22)	36.0%/35.4% (9/23)

JC= jaundice clearance

Table 6. Prognosis factors between different IgM and DNA combination groups.

	IgM (+)DNA (+)	IgM (+)DNA (-)	IgM (-)DNA (+)	IgM (-)DNA (-)
	n=15	n=10	n=15	n=50
Complete JC (n=44)	5	8*	7	24
Early JC (n=11)	1	3	0	7
Persistent JC (n=32)	3	6	7	16

***p=0.04**

JC= jaundice clearance

Figures

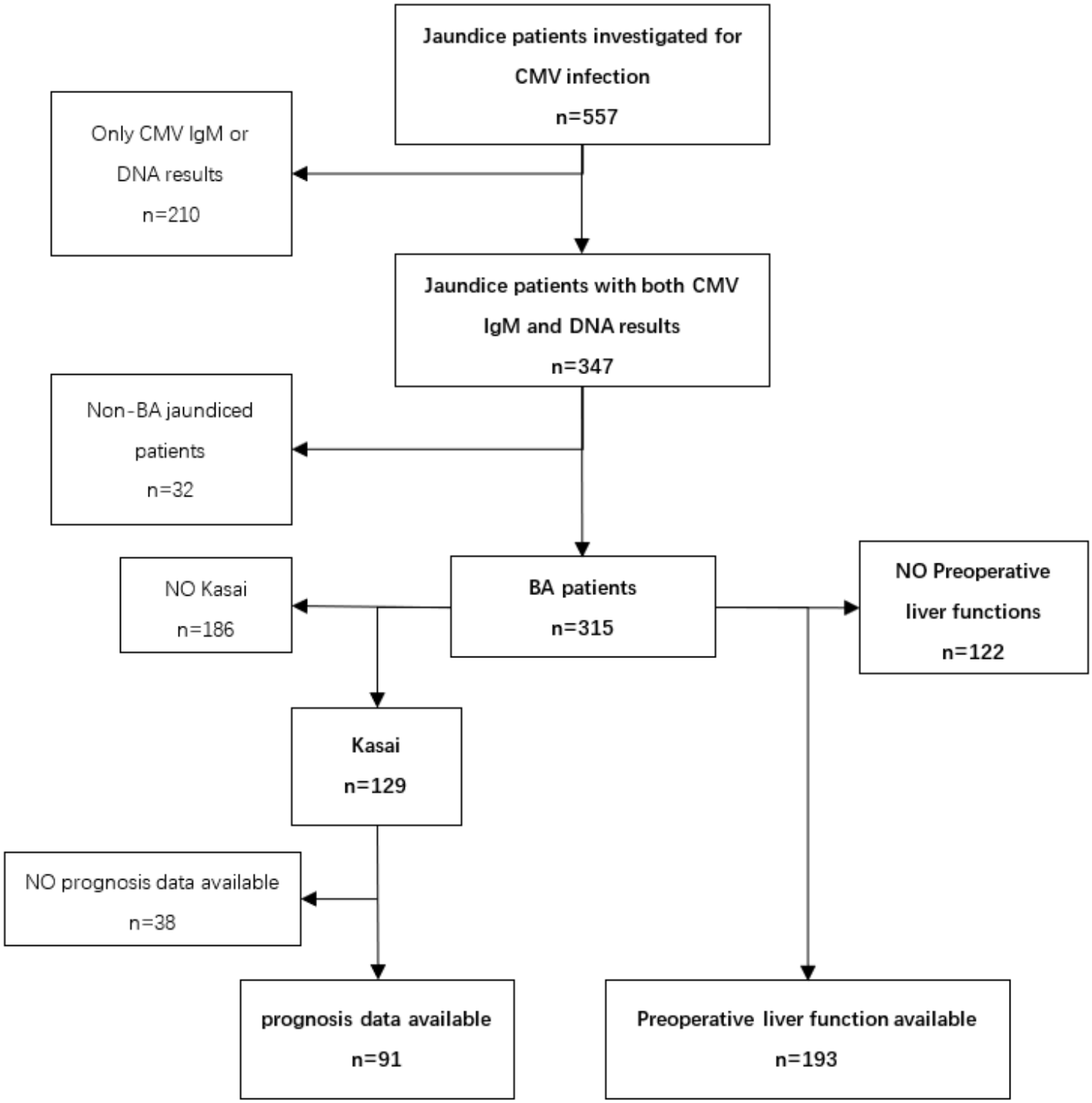

Figure 1

Total number of patients with obstructive jaundice, BA patients, patients with preoperative liver function and prognosis data.

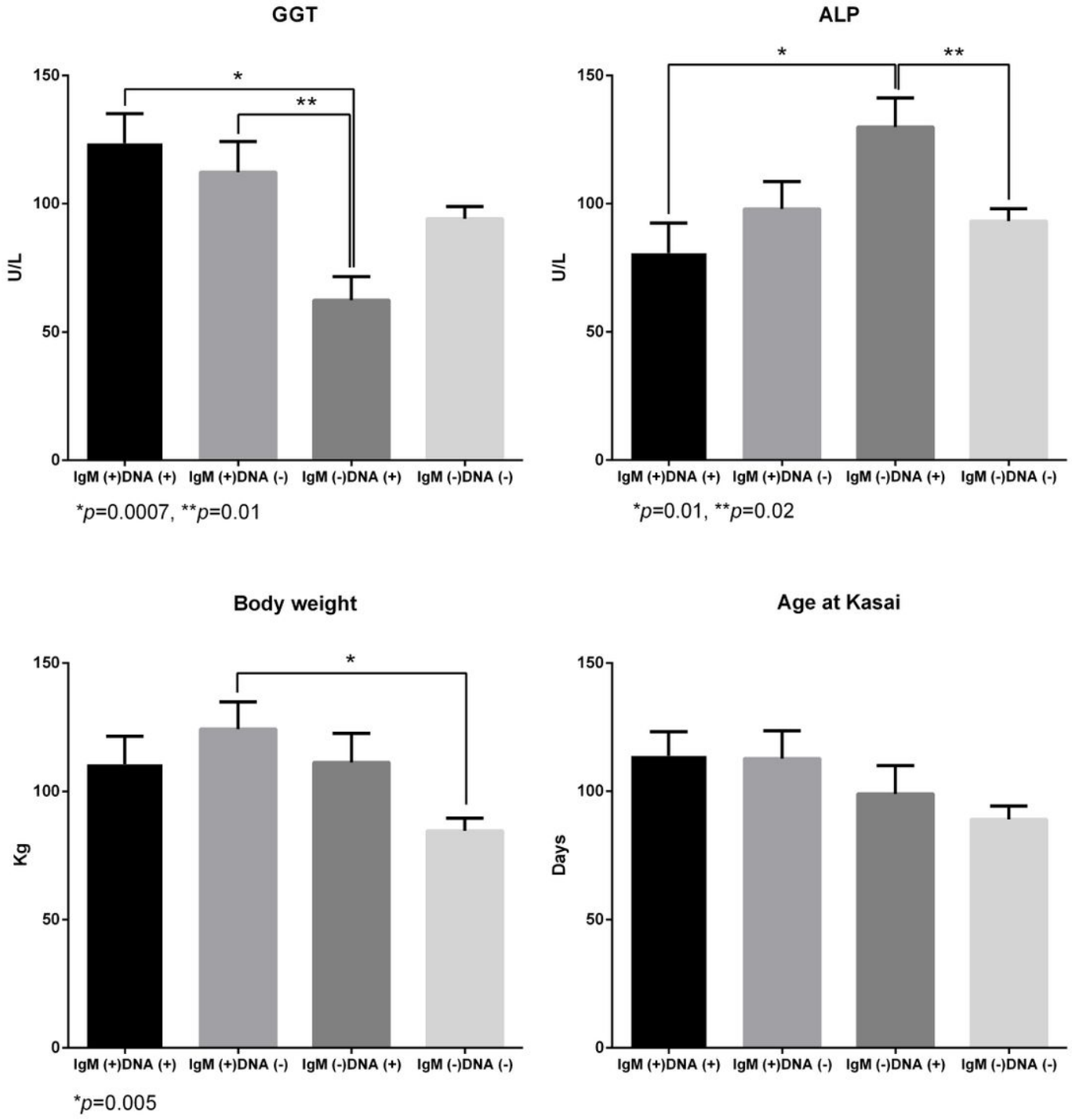

Figure 2

Comparation of GGT, ALP, preoperative bodyweight and age at Kasai between different IgM and DNA combination groups.