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# Accuracy of serology IGM anti-CMV and clinical manifestations as an alternative diagnostic of cytomegalovirus neonatal hepatitis

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## Abstract

**Background:** Main problem in cytomegalovirus (CMV) neonatal hepatitis diagnostic approach in developing country is the lack of virology examination. Serology is the most affordable examinations. **Methods:** This cross-sectional study was done at Dr. Soetomo Hospital, December 2011-April 2012. Inclusion criteria were jaundice, hepatomegaly, elevated direct bilirubin >2 mg/dLif total bilirubin <5 mg/dLor >20% if total bilirubin >5 mg/dL, and elevated aminotransferases >1.5 normal within first 3 months. The immunocompromised patient was excluded. McNemar and Kappa analyzed statistics. **Results:** From 30 enrolled-patients, 12 subjects were positive PCR CMV. The AUC was 0.968 (95% *confidence interval* 0.915-1.020; p<0.0001). The *cut-off* of IgM anti-CMV with best sensitivity (sn) and specificity (sp) value was 0.8 (sn 100%, sp 83.3%), 1.0 (sn 91.7%, sp 88.9%), 1.1 (sn 83.3%, sp 88.9%), 1.2 (sn 83.3%, sp 88.9%), dan 1.3 (sn 83.3%, sp 94.4%). **Conclusions:** The anti-CMV IgM serology examination has a high accuracy in the diagnosis of CMV neonatal hepatitis in a patient with clinical signs of jaundice, hepatomegaly, the increasing of direct bilirubin, and an increase in aminotransferase enzyme, with a cut-off value of 1.3 index units. The anti-CMV IgM serology examination can be applied in lieu of PCR for alternative diagnosis of CMV neonatal hepatitis.

**Keywords:** IgM anti-CMV, Clinical manifestations, Cytomegalovirus neonatal hepatitis, Diagnostic.

## INTRODUCTION

*Cytomegalo virus* infections still become the cause of most neonatal hepatitis. Main problems in CMV neonatal hepatitis approach in developing countries is the lack of virology examination facilities such as culture, PCR, and antigenemia which is actually recommended assets of diagnostic examination which recommended by experts. In developing countries, serology test become the most available and affordable examinations [1,2].

Range of CMV neonatal hepatitis clinical manifestations are very large, the most occurring cases are icterus, direct hyperbilirubinemia, hepatomegaly, and the increasing of aminotransferase enzyme [3-5]. Most are *self-limiting*; however, some can develop into becoming chronic, ongoing, even deadly about 2.8-13% case [6]. About 4-7% cases caused liver failures which need liver transplantations [6,8]. The accuracy of CMV neonatal hepatitis diagnostic is the key of successful therapies and determining prognosis [9,10]. The gold standard of CMV neonatal hepatitis diagnostic is virus detection in liver tissue or body fluids such as blood and urine by culture examinations. The using of serology in the approach of CMV neonatal hepatitis diagnostics is still in arguments due to lack of sensitivity and specificities, however in fact in many countries serology is still in use as first steps of suspected case before the examinations of PCR or antigenemia [11,12].

This research aimed to the bridge of diagnostic difficulties realities in a field with the importance of accuracy diagnostic approaches by researching the accuracy of serology IgM anti CMV examinations by using more limited inclusion criteria such as most occurring clinical manifests. This result of this research can be used as alternative diagnostics in CMV neonatal hepatitis.

## MATERIAL & METHODS

This research is a diagnostic trial, using *cross-sectional* approaches. Research was made in the outpatient clinic and pediatric ward of Dr. Soetomo Hospital Surabaya since January-June 2012. Research samples are inpatient in children care rooms or outpatients in pediatric policlinic of RSUD Dr. Soetomo Surabaya, which meet the inclusion and exclusion criteria. Inclusion criteria are patients which indicate clinic manifestations in the first three months as follow: icterus, hepatomegali, bilirubin direct level serum >2 mg/dL if bilirubin total <5 mg/dL or 20% from total bilirubin if total bilirubin >5 mg/dL, and the

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increasing of aminotransferase enzyme serum is 1.5 time than normal, that is ALT > 70 IU and AST > 55 IU, and also have signed the informed consent. The informed consent was obtained from parents to get their approval of this research. Patient history of treatment by using ganciclovir, HIV infections, miliary tuberculosis, malnutrition, and the history of using immunosuppressant medicine such as corticosteroid and Sito statistic drugs. The research flowchart was explained in the following figure.

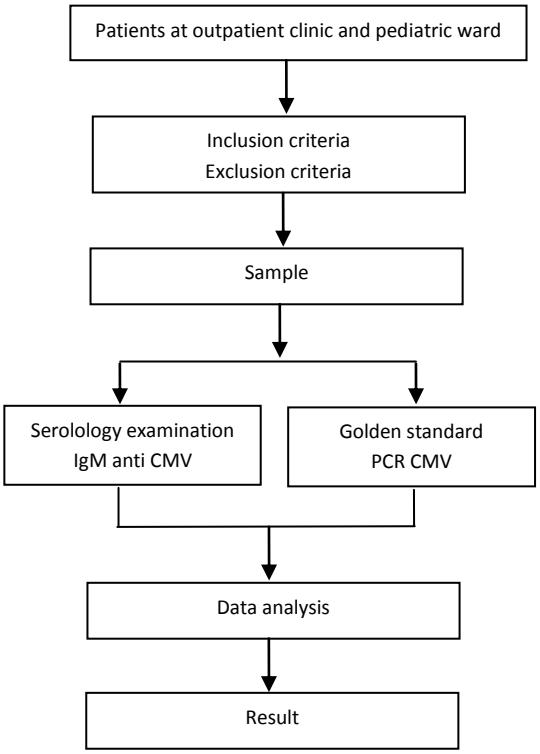


**Figure 1:** The flowchart of this research

The serology CMV examinations in this study done by using ELISA (*indirect*) in BalaiBesarLaboratoriumKesehatan Surabaya by using *cytolisaindec diagnostic kit* dari*INDEC* (sensitivity 94.4% and specificity 94.7%). Grade IgM anti CMV is positive if the result is ≥1.0 index units. The PCR CMV examination was held in ITD UNAIR using *QIAamp DNA kit* from *QIAGEN* (*primers* 5'-GGA TCC GCA TGG CAT TCA CGT ATG T-3' and 5'-GAA TTC AGT GGA TAA CCT GCG GCG A-3'). Those *primers* sequence have 100% sensitivity and 90% specificity [13].

Statistic analysis in the form of descriptive analysis to calculate sensitivity (sn), specificity (sp), positive predictive value (PPV), negative predictive value (NPV), likelihood ratio (LR), and prevalence (*pretest probability*) and inferential analysis in the form of Kappa Association and Mc Nemar Test with grade of significance (α) of 5%. Data was analyzed by SPSS program series 17.0. The ethical clearance was obtained by ethics committee Dr. Soetomo Hospital. The informed consent was obtained from parents to get their approval of this research.

**RESULTS**

30 patients become the samples of this research, most of them are men with *sex ratio* 1:1.7. Median age of all subjects is 3 months, minimal age 2 months and maximum age 5 months. All the subjects are less than 6 months of age and none of them are less than 2 months. Based on PCR results, the research subjects divided into two groups firstly PCR positive Group and secondly, PCR negative group. There are 12 subjects indicate positive PCR CMV or vertical CMV neonatal hepatitis diagnostics; therefore, prevalence of CMV neonatal hepatitis in this research is 40%. Statistically, there is no significant difference between age (*p* 0.593) and sex (*p* 0.750) with PCR result, as well as there is no significant difference between age (*p* 0.757) and sex (*p* 0.571) with IgM anti CMV result.

The description of clinical manifestations in this research was divided into several groups. Group classification according to clinical symptoms such as hepatomegaly divided into 2 groups which are 2-4 cm and > 4 cm, level ALT divided into 3 groups which are>70-<100 IU/L, 100-200 IU/L, and > 200 IU/L, and level AST divided 3 groups which are >55-<100 IU/L, 100-200 IU/L, and > 200 IU/L (table 1).

**Table 1:** Descriptions of clinical manifestations of subject research

| Clinical manifestations | PCR positive n = 12 | PCR negative n = 18 | *p* |
|---|---|---|---|
| Hepatomegaly (cm) | | | |
| 2-4, n | 0 | 7 | 0,014* |
| >4, n | 12 | 11 | |
| bilirubin total level (mg/dL) | | | |
| median (*range*) | 11,3 (7,2-15,7) | 12,5 (6,7-25,1) | 0,340 |
| bilirubin direct level (mg/dL) | | | |
| median (*range*) | 7,7 (4,2-12,4) | 9,0 (4,4-18,0) | 0,151 |
| ALT (IU/L) level | | | |
| median (*range*) | 86,5 (75-333) | 129,5 (80-677) | 0,238 |
| >70-<100, n | 7 | 4 | |
| 100-200, n | 4 | 10 | |
| >200, n | 1 | 4 | |
| AST (IU/L) level | | | |
| median (*range*) | 193,5 (99-322) | 162,5 (91-962) | 0,355 |
| >55-<100, n | 1 | 3 | |
| 100-200, n | 5 | 9 | |
| >200, n | 6 | 6 | |

**p*<0.05 (significant)

Icterus clinical symptoms is not analyzed due to all the subjects contain icterus. All PCR positive subjects include hepatomegali sized > 4 cm. Total median bilirubin in PCR positive group (11.3 mg/dL) is lower than PCR negative group (12.5 mg/dL), however, statistically the difference do not indicate significance. Subjects with the highest bilirubin level of 25.1 mg/dL have bilirubin direct level of 18 mg/dLand PCR negative result. Total median bilirubin total of all subjects is 11.5 (*range* 6.7-25.1) mg/dL. As well as the results of total bilirubin, direct median bilirubin in PCR positive group (7.7 mg/dL) is lower than PCR negative group (9.0 mg/dL), statistically the difference does not indicate

significance. The highest direct bilirubin level is 18 mg/dL with PCR negative results. Direct median bilirubin of all subjects is 7.9 (*range* 4.2 -18) mg/dL.

The distributions of ALT level indicate that median level ALT PCR negative group (129.5 IU/L) is higher than PCR positive group (86.5 IU/L), however statistically the difference is not significant. The highes ALT level is 677 IU/L, the subject has AST level of 775 IU/L and indicates PCR negative results. In PCR positive groups, the biggest part is in >70-<100 IU/L group, and followed by 100-200 IU/L group and > 200 IU/L group. Median level ALT of all subjects is 123 (*range* 75-677) IU/L. Different with ALT level, median level AST of PCR positive group (193.5 IU/L) higher than PCR negative group (162.5 IU/L), statistically the differences are insignificant. The highest level of AST is 962 IU/L, the subjects has ALT level of 213 IU/L and PCR negative result. In PCR positive groups, the biggest part is in > 200 IU/L groups, the followed by 100-200 IU/L groups and >55-<100 IU/L groups. Median level of AST of all subjects is 175 (*range* 91-962) IU/L.

In PCR positive groups, median score of IgM anti CMV is 1.8 (*range* 0.9-2.8) index units, the score is higher and more statistically significant ($p$<0.0001) than PCR negative group median which is only 0.4 (*range* 0.04-1.39) index unit and the whole subjects median at 0.8 (*range* 0.04-2.79) index units. The highest IgM anti CMV grade in this research is 2.79 index units, subject age is 3 months, male, having PCR positive result, and hepatomegaly of > 4 cm, total bilirubin of 14.08 mg/dL, direct bilirubin 12.39 mg/dL, ALT level of 179 IU/L, and AST level of 276 IU/L (figure 2).

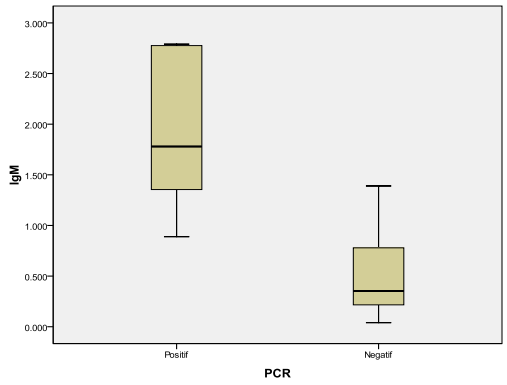


**Figure 2:** Distribution of IgM anti CMV value based on PCR results of $p$<0.0001. C*ut-off* grade was 1.0 index units

IgM anti CMV grades are analyzed by using ROC curve in order to obtain the highest sensitivity and specificity *cut-off* grade (figure 3). The ROC curve area dimension (AUC, *area under the curve*) is 0.968 with 95% *confidence interval* 0.915-1.020 and grade p<0.0001 (significant). From the ROC curve 5 grade of *cut-off* IgM anti CMV obtained with the highest sensitivity and specificity grade of 0.8 (sensitivity 100% and specificity 83.3%), 0.9 (sensitivity 91.7% and specificity 83.3%), 1.1 (sensitivity 83.3% and specificity 88.9%), 1.2 (sensitivity 83.3% and specificity 88.9%), and 1.3 (sensitivity 83.3% and specificity 94.4%). If using *cut-off* grade of 1.0 result of sensitivity 91.7% and specification 88.9% obtained(sensitivity and specificity of *commercial kit* is 94.4% and 94.7%).

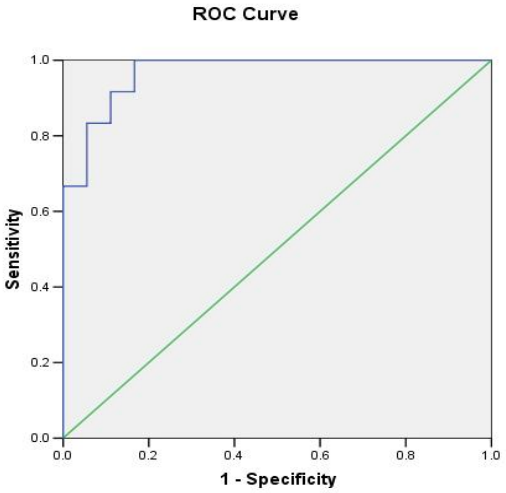


**Figure 3:** ROC curve of IgM anti CMV scores;size of *area under the curve* (AUC) is 0.968 with 95% *confidence interval* 0.915-1.020 and p<0.0001 (significant)

The accuracy of an examination is determined by sensitivity, spesifications, positive predictive value, negative predictive value, and likelihood ratio. The accuracy is said to be high if the grade of sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio is also high. The LR grades that consider to be significant is if it is higher than 10. The LR grade (positive) above 10 is obtained in grade *cut-off* of **1.3**. Table 2 below shows the accuracy of each *cut-off* grade of IgM anti CMV.

**Table 2:** The accuracy of IgM anti CMV Serology in CMV neonatal hepatitis diagnostic

| Variable (95% CI) | *cut-off*grade of IgM anti CMV | | | | | |
|---|---|---|---|---|---|---|
| | 0.8 | 0.9 | 1.0* | 1.1 | 1.2 | 1.3 |
| Sn (%) | 100 (69.9-100) | 91.7 (59.8-99.6) | 91.7 (59.8-98.1) | 83.3 (50.9-97.1) | 83.3 (50.9-97.1) | 83.3 (50.9-97.1) |
| Sp (%) | 83.3 (57.7-95.6) | 83.3 (57.7-95.6) | 88.9 (63.9-98.1) | 88.9 (63.9-98.1) | 88.9 (63.9-98.1) | 94.4 (70.6-99.7) |
| PPV (%) | 80 (51.4-94.7) | 78.6 (48.8-94.3) | 84.6 (53.7-97.3) | 83.3 (50.9-97.1) | 83.3 (50.9-97.1) | 90.9 (57.1-99.5) |
| NPV (%) | 100 (74.7-100) | 93.8 (67.7-99.7) | 94.1 (69.2-99.7) | 88.9 (88.9-98.1) | 88.9 (63.9-98.1) | 89.5 (65.5-98.2) |
| LR + | 6.0 (2.0-14) | 5.5 (1.9-16) | 8.3 (2.2-31) | 7.5 (1.9-28) | 7.5 (1.9-28) | 15 (2.2-102) |
| LR - | 0.0 (0.0-0.7) | 0.1 (0.0-0.7) | 0.1 (0.0-0.6) | 0.2 (0.1-0.7) | 0.2 (0.1-0.7) | 0,2 (0.1-0.6) |
| Prevalence (%) | 40 | 40 | 40 | 40 | 40 | 40 |
| *Pretest odds* | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| *Post-test odds* | 4.0 | 3.7 | 5.5 | 5.0 | 5.0 | 10 |
| Kappa Test ($p$)** | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| McNemar Test ($p$)*** | 0.250 | 0.625 | 1.000 | 1.000 | 1.000 | 1.000 |

Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; CI, *confidence interval*
**cut-off* from *commercial kit*; ***p*>0.05, no variation; ****p*<0.05, compatible

If using *cut-off* grade of 1.3 index units then below table 2x2 as shown in table 3 is acquired. There is 1 false positive subject and 2 false negative subjects.

**Tabel 3:** Table 2x2 between IgM anti CMV (cut-off 1.3 index unit) with PCR

| Serology | PCR | | Amount |
|---|---|---|---|
| | Positive | Negative | |
| IgM positive | 10 | 1 | 11 |
| IgM negative | 2 | 17 | 19 |
| | 12 | 18 | 30 |

**DISCUSSION**

From this research, we obtain prevalence of CMV neonatal hepatitis of 40%. In the previous study, the diagnostics of CMV neonatal hepatitis was using IgM anti CMV serology and the prevalence obtained by about 40-43% [14,15]. The youngest age of PCR positive groups is 2 months and nobody is under 2 month of age. This indicates that the age of the patient when firstly diagnosed as CMV neonatal hepatitis is 2-3 month. Therefore, the neonatal hepatitis cannot be determined due to congenital or perinatal because the age limit diagnostic to differentiate neonatal hepatitis congenital or perinatal is at the age of 2 weeks. Such condition is not quite different with other developing countries such as India and Brazil with the average age of patient firstly consult to approach neonatal hepatitis diagnostic is about 2-3 months [16,17]. In developed countries, the screening of CMV infections for newborn baby has been conducted therefore congenital cases is known and can have early therapy, besides TORCH examinations including CMV routine procedure in pregnancy examinations [18,19].

From clinical symptoms observed, there is a significant difference with PCR result on hepatomegaly. For other clinical symptoms (the increasing of bilirubin and aminotransferase), there is no significant difference betweenPCR positive group and PCR negative group; this means clinical manifestations only cannot differentiate between CMV neonatal hepatitis and non CMV.

Hepatomegaly is not only acquired by physical examination, but also from ultrasound. The dimensions of hepatomegaly in this research are divided into 2 groups which are 2-4 cm and>4 cm. The divide was made on the research of Braicu, in 2004, which grouping liver dimensions into 3 groups which was <2 cm, 2-4 cm, and >4 cm; group of<2 cm is the non-hepatomegaly group. The literature stated that a child identified as a hepatomegaly if liver felt more than 2 cm under right costa arcus [20,21]. All the PCR positive subjects in this research occurred to have hepatomegaly >4 cm. Hepatomegaly percentage in CMV neonatal hepatitis in other researches are 32% byLiberek (2002) [3], 32% byDistefano (2002) [22], 62.5% byBraicu (2004) [4], and 55.5% by Tasic (2005) [23]. The findings prove that hepatomegaly is considerable clinical symptom in CMV neonatal hepatitis. Even though clinical manifestation of CMV neonatal hepatitis is very large, however CMV infection should be considered if patients suffered persistent icterus which found hepatomegaly [3].

In PCR positive subject the direct bilirubin level increased until 68% from total bilirubin level. The pattern of cholestatic bilirubin increasing is the typical pattern of hyperbilirubinemia in CMV neonatal hepatitis although the cause of cholestatic hyperbilirubinemia is not caused by CMV infection. In the increasing of cholestatic bilirubin, the increasing bilirubin is the direct bilirubin. In CMV neonatal hepatitis, the bilirubin direct level can acquire more than 50% of bilirubin total level, therefore, the direct bilirubin level becomes the reference [14,24]. In this research, we use the limit of direct bilirubin of 2 mg/dLfollowing the cholestasis criteria from Dr. Soetomo Hospital. The limit of direct bilirubin level that became the reference is almost the same as other researches by about 1-2 mg/dL. NAPSGHAN specify cholestasis criteria with limit direct bilirubin of> 1mg/dL [25]. Other researcher such as Liberek (2002) and Oliveira (2002) using direct bilirubin level limit of> 1.5 mg/dLand> 2 mg/dL.

The increasing of ALT in PCR positive group indicates light increasing (1.5-2 times from normal), on the other hand, the increasing of AST indicates medium increasing (6-10 times from normal). The maximum ALT is 333 IU/L (7 times from normal grade, medium increasing) and maximum AST is 322 IU/L (10 times from normal grade, medium increasing). Those description are unlikely with the previous research which indicates the distributions of ALT as well as AST is around 1.5-2 times from normal grade that is ALT level of 70-100 IU/L, AST level of 55-100 IU/L, and not more than 200 IU/L. Different from Shibata, in 2005, it found that the peak amino transferase can reach 700 IU/L (15 times from normal grade, medium-high increasing). Unfortunately, Shibata did not explain regarding the height of the mentioned aminotransferase level. The degree of the increasing of aminotransferase can be classified as low, medium, and high.[26]The large classifications of amino transfeases level used are classifications of Giannini (2005) and Thapa (2007). Giannini classification is called low if <5 times from normal grade, medium if 5-10 times from normal grade, and high of >10 times from normal grade; whereas Thapa classification, is called low if 1-3 times from normal grade, medium if 3-20 times from normal grade, and high if >20 times from normal grade [27,28].

The increasing of aminotransferase (78-100%) is a clinical symptoms that commonly found than hepatomegaly (32-62.5%) and the increasing of direct bilirubin (60-80%). The increasing of aminotransferase could be an early symptom of CMV neonatal hepatitis [19, 29]. Commonly, aminotransferase test do not provide specific informations regarding certain liver illness diagnostics, however the increasing of ALT is more specific for liver failure. In research conducted by Chiba (1975), there is significant positive correlation between the findings of CMV virus in the subject with the increasing of ALT [29].

We found in this research that there is gap between the dimension of hepatomegaly with the increasing of ALT level (hepatomegaly>4 cm versus ALT >70-<100 IU/L). The CMV infections itself do not create massive malfunction in liver thus there is only light increasing of aminotransferase and light hepatomegaly happened. Braicu (2004) categorized hepatomegaly criteria as CMV neonatal hepatitis are <2 cm (40%), 2-4 cm (40%), and >4 cm (20%) [19]. The explanation of the gap that the degree of aminotransferase increasing is not correlated with the clinical measurement of hepatomegaly [20].

The highlight result of this research indicates that the serology examinations indicate by IgM anti CMV have high accuracyin CMV neonatal hepatitis diagnostics. The accuracy valued by sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio. A diagnostic examinations said to be having high accuracy if the diagnostic parameters created is also having a high grade [30]. If the CMV serology using determined *cut-off* value of 1.0 index units, we found the sensitivity of 91.7%, specificity of 88.9%, positive prediction value of 84.6%, negative prediction value of 94.1%, positive likelihood ratio of 8.3, and negative likelihood ratio of 0.1 (whereas sensitivity and specification factory 94.4% and 94.7%) acquired. Based on that results, the accuracy of IgM anti CMV with *cut-off* 1.0 is high enough. We also conducted analysis using ROC curve to obtain the best *cut-off* value by arrangement between sensitivity and specificity. The best *cut-off* value is the highest point at the upper-left diagonal line on ROC curve.[31]

The width of AUC is 0.968 with 95% *confidence interval* 0.915-1.020 (p<0.0001). The width of AUC is considered large and significant. From ROC curve, we also obtained 5 *cut-off* value of IgM anti CMV with the highest sensitivity and specificity value that is 0.8, 0.9, 1.1, 1.2, and 1.3 index units. From the calculation of the *cut-off* we obtained result as follow *cut-off* 0.8 (sensitivity 100% and specificity 83.3%), 0.9 (sensitivity 91.7% and specificity 83.3%), 1.1 (sensitivity 83.3% and specificity 88.9%), 1.2 (sensitivity 83.3% and specificity 88.9%), and 1.3 (sensitivity 83.3% and specificity 94.4%). In ROC curve, the farthest point from the diagonal line is in *cut-off* 1.0 and 1.3 index units.

In the arrangement of the under, mentioned sensitivity and specificity we must pay attention to the main goal of the conducted serology examination.In the case of CMV neonatal hepatitis, the importance of serology examinations is to approach the diagnostics, therefore the examinations with high sensitivity value (to avoid false negative) and also high specificity (to avoid false positive) is required. The *cut-off* value which are already calculated (0.8, 0.9, 1.0, 1.1, 1.2, and 1.3) have high enough sensitivity and specificity by about 83.3-100%.

The 100% sensitivity result gained in*cut-off* value of 0.8 index units. If we use a sensitive test, the normal result gained (negative result) can be used to get rid of the illness as we usually use in a screening test. The screening requires test with high sensitivity to be applied in asymptomatic patients. However, using serology CMV as a screening test requires higher diagnostic confirmation which filled by PCR and antigenemia. In limited facility like in Indonesia especially in Surabaya, the PCR or antigenemia are not always available. In our reference journals we also found out that the serology is still conducted as the early step of CMV neonatal hepatitis diagnostics, and no matter how much the titer is, it always be confirmed with PCR or antigenemia [14, 26]. Funato and Shibata conducted CMV neonatal hepatitis screening test with qualitative PCR, if the result was positive then it was continued with quantitative PCR (*cut-off* 10 copies/ µg DNA) to start the therapy [14, 26].

The highest specificity result of 94.4% gained in *cut-off* value of 1.3index units. In the chosen of diagnostic examination for the purpose of approaching diagnosis the high specificity is required. In a specific test, the abnormal result obtained (positive test result) could be used to determine the existence of illness. High specificity will avoid false positive which in CMV hepatitis case will avoid ganciclovir therapy which is not required [19].

The likelihood ratio (LR) component is also assessment guidelines of the accuracy diagnostics of an examination. The bigger of LR value (positive) indicates, the bigger the examinations separate the ill subjects and the non-ill ones. The LR value (positive) which considered important is 10 or more [30]. In this research, the LR value (positive) above 10 acquired in *cut-off* value of 1.3 index units.

The decision-making using a kind of diagnostic testing also indicated from the ability of diagnostic test equipments increasing the pretest probability value into post-test probability [30]. In this research, the biggest cut-off value which increase pre-post probability value is cut-off value of 1.3 index units (pretest probability 40% and post-test probability 90,9%). On second place is cut-off value of 1.0 index units (pretest probability 40% and post-test probability 84,6%).

The threshold model from Puker and Kassirer which described decision-making model based on pretest probability mentioned that if pretest probability diagnostic test 25-65% then it is better to conduct diagnostic test [32]. In this research, the obtained pretest probability is 40% therefore the examination of serology IgM anti CMV is better conducted to patient with inclusion criteria mentioned in this research. It makes the direction of the controversy either the capability or incapability of serology examination conducted as alternative diagnosis for CMV neonatal hepatitis.

In this research, there are 2 patients with negative false result and 1 patient with positive false result. In negative false result, it could explainthatIgMhas not been produced due to immature infant system [26]. Virus Epstein barr can stimulate B cells to produce antibodies and the mentioned antibodies will be detected as anti CMV antibodies. This explains the positive false results in this research [12, 32].

The cut-off value of anti-CMV IgM in the diagnosis of CMV neonatal hepatitis are recommended based on this study was1.3 index units, it is based on the farthest point from the diagonal line on the ROC curve, has a positive LR value of 10 and it is the greatest among the other cut-off, and also can raise the value of pretest probability into post-test probability greatest among the other cut-off. That mentioned cut-off value was set at the likely patient who had jaundice, hepatomegaly, increasing direct bilirubin level, and an increasing aminotransferases level.

**CONCLUSION**

The anti-CMV IgM serology examination has a high accuracy in the diagnosis of CMV neonatal hepatitis in patient with clinical signs of jaundice, hepatomegaly, the increasing of direct bilirubin, and an increase in aminotransferase enzyme, with a cut-off value of 1.3 index units. The anti-CMV IgM serology examination can be applied in lieu of PCR for alternative diagnosis of CMV neonatal hepatitis.

The possibility of dual infection in cases of CMV neonatal hepatitis can not bee eliminated therefore the examination to search for a etiology beside CMV of neonatal hepatitis also needs to be carried out.

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**REFERENCES**

1. Heathcote EJ. Diagnosis and management of cholestatic liver disease. ClinGastrHep 2007;5:776-82.
2. Gandhi MK, Khanna R. Human Cytomegalovirus: Clinical aspects, immune regulation, and emerging treatments. Lancet Infect Dis 2004;4:725-38.
3. Liberek A, Rytlewska M, Sidorkiewicz AS, 2002. Cytomegalovirus disease in neonates and infants - clinical presentation, diagnostic and therapeutic problems - own experience. Med Sci Monit;8:815-20.
4. Braicu S, Nicola R. Neonatal hepatitis with cytomegalovirus. J Pediatrului 2004;7:1-5.
5. Oliveira N, Kanawaty F, Costa SC, Hessel G. Infection by cytomegalovirus in patients with neonatal cholestasis. ArqGastroenterol 2002;39:132-6.
6. Chang MH, Hsu HC, Lee CY, 1987. Neonatal hepatitis: a follow-up study. J PediatrGastroenterol Nutr;6:203-7.
7. Min YH, Fang ZX, Jing S. Current state of clinical diagnosis and treatment of infantile cytomegalovirus hepatitis. Chin J Integr Med 2010;16:87-91.
8. Lee WS, Chai PF, Boey CM. Aetiology and outcome of neonatal cholestatic in Malaysia. Singapore Med J 2010;51:434-39.
9. Yaghobi R, Zamami S. The molecular and antigenic tissue impact of viral infections on liver transplant patients with neonatal hepatitis. Int J Org Transplant Med 2011;2:108-15.
10. Juffrie M, Mulyani NS. Kolestasispadabayi. Dalam: Juffrie M, Mulyani NS, penyunting. Modulkolestasis. Edisipertama. Jakarta;UKK gastro-hepatologiIkatanDokterAnak Indonesia 2009:10-33.
11. Pawlowska J, Swiatkowska E, Gliwicz D. The role of cytomegalovirus infection in pathogenesis of neonatal cholestasis. Hepatology 2010;6:25-9.
12. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Micro Rev 2002;15:680-715.

13. Soetens O, Fellous CV, Foulun I. Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. J ClinMicrobiol 2008;46:943-6.
14. Funato T, Satou N, Abukawa D. Quantitative evaluation of cytomegalovirus DNA in infantile hepatitis. J VirHep 2001;8:217-22.
15. Brandao MA, Andrade PD, Costa SC. Cytomegalovirus frequency in neonatal intrahepatic cholestasis determined by serology, histology, immunohistochemistry, and PCR. World J Gastroenterol 2009;15:3411-16.
16. Aanpreung P, Laohapansang M, Ruangtrakool R, Kimhan J. Neonatal cholestasis in Thai infants. J Med Assoc Thai 2005;88:9-14.
17. Chakravarti A, Kashyap B, Matlani M. Cytomegalovirus infection: an Indian perspective. Ind J Med Microbiol 2009;27:3-11.
18. Lazzarotto T, Guerra B, Spezzacatena P. Prenatal diagnosis of congenital cytomegalovirus infection. J ClinMicrobiol 1998;36:3540-4.
19. Ozkan TB, Mistik R, Dikici B, Nazlioglu HO. Antiviral therapy in neonatal cholestatic cytomegalovirus hepatitis. BMC Gastroenterology 2007;7:1-5.
20. Wolf AD, Lavine JE. Hepatomegaly in neonates and children. Pediatr Rev 2000;21:303-10.
21. Lynele MB, William FB. Manifestations of liver diasease. Dalam: Kliegman RM, Behrman RE, Stanton BF, Schor NF, penyunting. Nelson textbook of pediatrics. 19th edition. Philadelphia;Elsevier Saunders 2011:1374-8.
22. Distefano AL, Alonso A, Martin F, Pardon F. Human cytomegalovirus: detection of congenital and perinatal infection in Argentina. BMC Pediatrics 2004;4:1-8.
23. Tasic G, Djordjevic DS, Dinic M. Cytomegalovirus infection in infants with hepatosplenomegaly. Med Biol 2005;12:154-8.
24. Tiker F, Tarcan A, Kilicdag H, Gurakan B. Early onset conjugated hyperbilirubinemia in newborn infants. Indian J Pediatr 2006;73:409-12.
25. NAPSGHAN. Guideline for the evaluation of cholestatic jaundice in infant: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2004;39:115-28.
26. Shibata Y, Kitajima N, Kawada J. Association of cytomegalovirus with infantile hepatitis. MicrobiolImmunol 2005;49:771-7.
27. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Can Med Ass J 2005;172:367-79.
28. Thapa BR, Walia A. Liver function tests and their interpretations. Ind J Ped 2007;74:663-71.
29. Chiba S, Hori S, Kawamura N, Nakao T. Primary cytomegalo virus infection and liver involvement in early infancy. Tohoku J Exp Med 1975;117:143-51.
30. Sastroasmoro S. Telaahkritismakalahkedokteran (2). Dalam: Sastroasmoro S, Ismael S, penyunting. Dasar-dasarmetodologipenelitianklinis. Edisi ke-4. Jakarta;SagungSeto 2011:469-80.
31. Sackett DL. The interpretation of diagnostic data. Dalam: Sackett DL, Haynes RB, Guyatt GH, Tugwell P, penyunting. Clinical epidemiology. A basic science for clinical medicine. Second edition. Boston;Little Brown 1991:69-152.
32. Lang D, Vornhagen R. Cross-reactivity of Epstein-barr virus specific immunoglobulin M antibodies with cytomegalovirus antigen containing glycine homopolymers. ClinDiagn lab Immunol 2001;8:747-56.