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Study the correlation between the Mother-to-child Transmission Risk Factors in chronic hepatitis B virus infection pregnant women in Vietnam by HCA method

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BACKGROUND&AIMS: Hepatitis B virus (HBV) infection remains a major public health problem. The interaction between HBV and the host inflammatory response is an important factor contributing to liver damage and disease development. We compared the correlation between the subclinical index and PBMCs concentration in two groups of pregnant women (HBsAg positive), which are different in HBV DNA concentration in Vietnam. **METHODS:** The Hierarchical cluster analysis (HCA) was run with 20 different clustering methods on data collected from 80 Vietnamese pregnant women and their babies (60/80 cord blood). **RESULTS:** When maternal viral load is higher than 5x10^7 copies/ml, the risk of being HBsAg positive in cord blood is 123% (RR=2.23 [1.48,3.36]); when viral load is lower than this baseline, this risk is decreased by 55% (RR=0.45 [0.30,0.67]). (p<0.001). In the high viral load group (HBV DNA ≥ 5x10^7 copies/ml), a strong correlation between CBMCs with serum maternal Hemoglobin concentration, maternal platelet and maternal ALT. Their R values are: -0.88, 0.82, and 0.84 with p=8.97E-03, 2.41E-02 and 1.75E-01, respectively. **CONCLUSIONS:** We found a significant correlation shift of subclinical index between the two groups, which may be important in diagnosing pregnant women with chronic hepatitis B virus infection.

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Keywords: Chronic Hepatitis B Virus Infection; Liver; Pregnant Women; Cord blood; PBMCs (Peripheral Blood Mononuclear Cells); subclinical index

Abbreviations used in this paper: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC**,** umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity

HBV Mother-to-child transmission (MTCT) can occur by transplacental infection or blood-to-blood contact around or after delivery, and it accounts for a significant proportion of chronic HBV infections worldwide[1]. In high-prevalence nations like Vietnam, MTCT remains the most significant transmission mode. Children who acquire a chronic infection have a 40% lifetime chance of dying due to HBV infection. Antiviral treatment to lower high virus loads and immuno-prophylaxis with anti-HBV immunoglobulin shortly after delivery coupled with active HBV immunization of neonates can minimize the incidence of MTCT, although these measures are seldom used in most countries.[2] The studies of high-risk variables linked to MTCT in HBV patients lack in-depth investigation, and the association between Hepatitis B virus DNA in maternal serum and cord blood is unknown. We set up a Clustering study to find the possible link between HBV infection in pregnant women and cord blood in order to give a clinical reference marker for prenatal surveillance and postnatal management, as well as to enhance the prevention of HBV MTCT

Clustering is a machine learning approach for identifying subgroups of homogeneous individuals who share characteristics with other groups or clusters [3,4]. Clustering is used in the medical field for image processing, document classification, and group building although it is required to handle the interaction of multiple variables [5,6] A single link between the subclinical index and pregnancy has been shown in several research. There are some of the categories discovered in the pregnancy-related literature, for example: Hypertension[7,8], preeclampsia[9,10], fetal growth restriction[11], miscarriage [12], the incidence of pregnancy termination linked to demographics[13], nutritional and birth control related to mother education, cleanliness, and nutrients [14]. However, there have been few studies on clustering to better define the link between the subclinical index during pregnancy, particularly in the case of HBV infection and pregnancy problems. This study used cluster analysis to look for probable subgroups within a well-defined cohort of HBsAg-positive first-trimester pregnant women. This research might potentially aid in the identification of possible MTCT risk factors.

Patients and Methods

Study Population

From 2020 to 2021, we performed a pilot study on pregnant women who had frequent check-ups and deliveries at Thai Nguyen National Hospital in Vietnam, and 80 HBsAg positive pregnant women were finally included in the study. The "Study on the risk of mother-to-child transmission of Hepatitis B virus with T cell immunity and gene variation in pregnant woman HBsAg(+)" was recognized and accepted by the

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Institution Review Board for Ethics in Biomedical Research of Hanoi Medical University's institutional ethics council (Number NCS22/HMU-IRD), and all participants' rights were protected.

We collected samples and performed clinical surveillance on pregnant women who had chronic hepatitis B virus infection. We want to create a hepatitis B patient cohort that includes pregnant women (HBsAg+ for >6 months) and collect various bio-samples in order to conduct an HCA (hierarchical analysis) study to determine predictors of HBV MTCT. Patients will be provided treatment based on WHO standards if their HBV-DNA level is greater than 200.000 IU/ml.

Diagnostic criteria

We collected the clinical data of the of pregnant women, who have HBsAg positif, including the general conditions: age of delivery, gestational age, pre-pregnancy body mass index (BMI), prenatal BMI, the number of prenatal check-ups, education level, previous healthcare history, HBV infection; the laboratory investigations: ALT, AST, HBV DNA and use of antiviral therapy. We also considered pre-eclampsia, chronic hypertension, abortion, placental abruption, hyperthyroidism, gestational diabetes mellitus, hypertension during pregnancy, intrahepatic cholestasis of pregnancy (ICP), and other maternal problems. We retrospectively analyzed and explored the correlation between HBV infection status and the preclinic.

Isolation of Peripheral Blood Mononuclear Cells

EDTA maternal blood and umbilical cord blood was collected; Serum/plasma samples were analyzed for viral markers. Peripheral blood mononuclear cells (PBMC) and umbilical cord blood mononuclear cells (CBMC) were isolated from Heparin blood by a standardized and harmonized protocol[15] and stored in liquid nitrogen for further analysis and / or transport.

Statistic and clustering analysis

The data collection, storage tools, and analysis of this study were conducted using the R.4.1.0 packages tools. The groups were compared using the independent sample t-test, the Pearson's chi-square test, and Fisher's exact test to clarify the differences. The relative risk ratio (relative RR), risk ratio (RR), odds ratio (OR), and 95% confidence interval (CI) were calculated. $P < 0.05$ indicated the significant difference. Cluster analysis was performed on the correlation test results in R.4.1.0 environment. K-means was optimized following three methods: Average Silhouette, Elbow, and Gap statistic. The cluster analysis carried out did not have any missing values.

Results

Correlation between the Maternal PBCMs concentration with the maternal viral load

The correlation R measures the strength of the linear relationship between two quantitative variables. Pearson R formular is:

$$\boldsymbol{R} = \frac{\mathbf{1}}{\boldsymbol{n-1}} \sum \left(\frac{\boldsymbol{x_i - \bar{x}}}{\boldsymbol{S_x}}\right)\left(\frac{\boldsymbol{y_i - \bar{y}}}{\boldsymbol{S_y}}\right) \quad [16]$$

Equation 1: The point biserial correlation coefficient. R: Pearson correlation coefficient, x and y: two vectors

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of length i and j.

R is always a number between -1 and 1. R > 0 indicates a positive association. R < 0 indicates a negative association. Values of R near 0 indicate a very weak linear relationship. The strength of the linear relationship increases as R moves away from 0 toward -1 or 1. The extreme values R = -1 and R = 1 occur only in the case of a perfect linear relationship.[16]

R score	Mat AST	Mat ALT	Mat HBV DNA	Mat PBMCs Concentration
Mat AST	1.00	0.87	0.27	-0.12
Mat ALT	0.87	1.00	0.36	-0.03
Mat HBV DNA	0.27	0.36	1.00	-0.23
Mat PBMCs Concentration	-0.12	-0.03	-0.23	1.00
P value	**Mat AST**	**Mat ALT**	**Mat HBV DNA**	**Mat PBMCs Concentration**
Mat AST	NA	0.00E+00	1.26E-02	2.95E-01
Mat ALT	0.00E+00	NA	1.01E-03	7.60E-01
Mat HBV DNA	1.26E-02	1.01E-03	NA	4.15E-02
Mat PBMCs Concentration	2.95E-01	7.60E-01	4.15E-02	NA

Table 1. R score (upper) and P value (lower) from Pearson's correlation test. The color-coded correlation factors between the subclinical indexes including levels of AST, ALT, concentration of PBMCs, HBV DNA concentration in the mother blood. The color value of the cells is proportional to the strength of the associations, ranging from red (negative correlations) to blue (positive correlations). The strength of the correlation is indicated in the color scale. Method: Pair-wise Pearson correlation coefficients. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate; Mat: Mother or Maternal.

R is always a number between -1 and 1. R > 0 indicates a positive association. R < 0 indicates a negative association. Values of R near 0 indicate a very weak linear relationship. The strength of the linear relationship increases as R moves away from 0 toward -1 or 1. The extreme values R = -1 and R = 1 occur only in the case of a perfect linear relationship.[16] Taking the data from 80 pregnant women, we found the negative association between their viral load versus their PBMCs concentration (R = -0.23, p=0.04) and positive association between their viral load versus their ALT values (R = 0.36, p=0.001). (Table 1)

Relative risk ratio shows the predicted values affect on cord blood HBsAg positive probability

In 60 Vietnamese CHB pregnant women, 32 (53.3%) cord-blood samples were HBsAg positive, and 28 (46.7%) HBsAg negative. We fit a logistic regression model to predict the subclinical values, which link with 50:50 probabilities that HBsAg in cord blood is positive. Two variables have a positive association when above-average values of one tend to accompany above-average values of the other, and when below-average values also tend to occur together. Two variables have a negative association when above-average values of one tend to accompany below-average values of the other.[16] (Table 1, Supplementary figure 1).

	Cutoff50 for HBsAg positive probability in cord blood	The direction of the relationship between the two variables.	Figure Name (Supplementary figure 1)
Mat PBMCs Concentration	8.03E+06 (cells/ml)	negative	A
Mat HBV DNA	5.40E+07 (copies/ml)	positive	B

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Mat Platelet	317.89 (x10^3cells/ml)	negative	C
Mat ProthrombininS	11.00 (Second)	positive	D
CBMC concentration	6.64E+06(cells/ml)	positive	E
Mat Hb	128.53 (g/l)	negative	F
Mat RBC	5E+06 (cells/ml)	negative	G
Mat Creatinin	37.46 (umol/l)	positive	H
Mat AST	14.15 (U/l)	positive	I
Mat ALT	43.34 (U/l)	negative	K
Mat Prothrombinin Percent	76.34 (%)	positive	L

Table 1. Relative risk ratio. Predicted values linked with a 50:50 probability that HBsAg is detectable in cord blood (Cutoff50). Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

We calculate the risk ratio and odds ratio following the new value indications (table 2) with two groups, HBsAg Cord blood (CB HBsAg), positive and negative. When maternal viral load is higher than $5x10^7$ copies/ml, the risk of being HBsAg positive in cord blood is 123% (RR=2.23 [1.48,3.36]); when viral load is lower than this baseline, this risk is decreased by 55% (RR=0.45 [0.30,0.67]). (p<0.001). (Table 2, Supplementary Table 5).

		CB HBsAg positive (N=32)	CB HBsAg negative (N=28)	RR	95%CI	OR	95%CI	Chisq	Pr>chisq
Mat PBMCs Concentration (Cells/ml)	>=8.03e+06	6	7	0.83	[0.44,1.58]	0.69	[0.20,2.38]	0.34	0.56
	<8.06e+06	26	21	1.2	[0.63,2.28]	1.44	[0.42,4.96]		
Mat HBVDNA (copies/ml)	>=5*10^7	15	2	2.23	[1.48,3.36]	11.47	[2.32,56.65]	11.61	<0.001 ***
	<5*10^7	17	26	0.45	[0.30,0.67]	0.09	[0.02,0.43]		
Mat Platelet (x10³ cells/ml)	>=317.89	2	2	0.93	[0.34,2.56]	0.87	[0.11,6.59]	0	1
	<317.89	30	26	1.07	[0.39,2.94]	1.15	[0.15,8.78]		
Mat Prothrombin in S (Second)	>=11	29	25	1.07	[0.46,2.48]	1.15	[0.21,6.27]	0	1
	<11	3	3	0.93	[0.40,2.15]	0.86	[0.16,4.66]		
CBMC concentration (cells/ml)	>=6.64e+06	22	22	0.8	[0.49,1.29]	0.6	[0.19,1.94]	0.73	0.39
	<6.64e+06	10	6	1.25	[0.77,2.02]	1.67	[0.52,5.38]		
Mat Hb (g/l)	>=128.53	11	12	0.84	[0.51,1.40]	0.7	[0.25,1.99]	0.46	0.5
	<128.53	21	16	1.19	[0.71,1.98]	1.43	[0.50,4.07]		
Mat RBC (cells/ml)	>=5e+06	2	3	0.73	[0.24,2.20]	0.56	[0.09,3.59]	0.024	0.88
	<5e+06	30	25	1.36	[0.45,4.10]	1.8	[0.28,11.64]		
MatCreatinin (umol/l)	>=37.46	28	23	1.24	[0.57,2.67]	1.52	[0.37,6.33]	0.047	0.83
	<37.46	4	5	0.81	[0.37,1.75]	0.66	[0.16,2.73]		
MatAST (U/l)	>=14.15	29	23	1.49	[0.59,3.76]	2.1	[0.45,9.73]	0.34	0.56
	<14.15	3	5	0.67	[0.27,1.70]	0.48	[0.10,2.20]		
MatALT (U/l)	>=43.34	4	1	1.57	[0.94,2.62]	3.86	[0.40,36,75]	0.61	0.44
	<43.34	28	27	0.64	[0.38,1.06]	0.26	[0.03,2.47]		
Mat Prothrombin in Percent (%)	>=76.34	31	27	1.07	[0.26,4.36]	1.15	[0.07,19.25]	0	1
	<76.34	1	1	0.94	[0.23,3.82]	0.87	[0.05,14.60]		

Table 2. Risk Ratio to be HBsAg positive cord blood of each factor (with their boundary value). Abbreviations: RR, Risk ratio; OR, Odds ratio; 95%CI, 95% confidence interval; chisq; chi square value; HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrfombininS: Prothrombin time in

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second, ProthrombininPercent: Prothrombin % activity. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Correlation between CBCMs concentration with the maternal subclinical index

There are the significant negative association (R<0 and p<0.05) and the positive association (R>0 and p<0.05) between the parameters. The direction of the linear relationships are also different between the two viral load groups. (Figure 2, Table 3, and Supplementary tables 1-4).

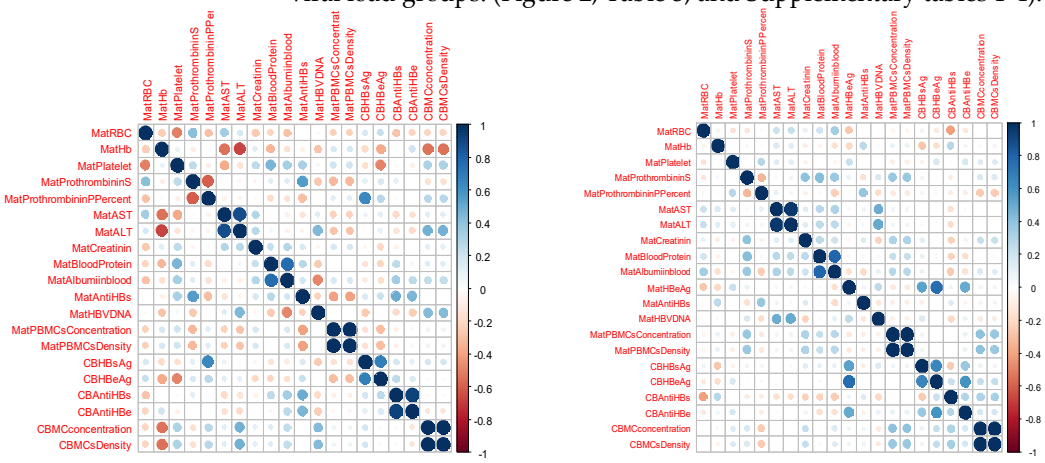

Figure 2. Correlation matrix between biomarkers depicted as a heatmap, two groups HBV-DNA ≥ $5x10^7$ copies/ml (left) and HBV-DNA < $5x10^7$ copies/ml (right). Heat map represents the color-coded correlation factors between all the subclinical indexes, including Prothromibin, AST, ALT, RBC, Hb in mother blood; concentration and density of PBMCs, the status of HBeAg, AntiHBs in Cord and Mother blood. The color value of the cells is proportional to the strength of the associations, ranging from red (negative correlations) to blue (positive correlations). The strength of the correlation is indicated in the color scale (at the right of the panel). Pair-wise Pearson correlation coefficients are shown in Supplementary Table 1-4. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

	HBVDNA≥$5x10^7$copies/ml		HBVDNA<$5x10^7$copies/ml	
	R	p	R	p
Mat RBC - Mat HBVDNA	0.779	3.89E-02	0.088	5.29E-01
Mat RBC - Mat PBMCs Concentration	-0.863	1.24E-02	0.019	8.94E-01
Mat RBC - Mat PBMCs Density	-0.863	1.24E-02	0.019	8.91E-01
Mat RBC – CB HBsAg	-0.231	6.18E-01	-0.305	2.66E-02
Mat Hb - Mat Prothrombin in Second	-0.850	1.55E-02	0.048	7.35E-01
Mat Hb - Mat AntiHBs	-0.779	3.89E-02	0.257	6.36E-02
Mat Hb – CB AntiHBs	-0.766	4.44E-02	0.254	6.65E-02
Mat Hb – CBMCs Concentration	-0.880	8.97E-03	-0.027	8.46E-01
Mat Hb – CBMCs Density	-0.880	9.05E-03	-0.044	7.56E-01
Mat Platelet – Mat Creatinin	0.797	3.81E-02	-0.070	6.20E-01
Mat Prothrombin in Second – Mat Prothrombin Percentage	0.565	1.88E-01	-0.745	1.52E-10
Mat Prothrombin in Second – Mat AntiHBs	0.766	4.48E-02	0.158	2.58E-01
Mat Prothrombin in Second – CBMCs Concentration	0.819	2.41E-02	-0.114	4.15E-01
Mat Prothrombin in Second – CBMCs Density	0.819	2.41E-02	-0.115	4.15E-01
Mat Prothrombin Percentage – CB HBsAg	0.969	3.19E-04	-0.065	6.41E-01
Mat Prothrombin Percentage – CB HBeAg	0.969	3.19E-04	-0.212	1.28E-01
Mat AST - Mat ALT	0.923	3.03E-03	0.896	<0.00001

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Mat AST – Mat HBV DNA	0.690	2.51E-02	0.605	1.61E-06
Mat ALT – Mat AntiHBs	0.816	5.18E-03	-0.019	8.92E-01
Mat ALT – CBMCs Concentration	0.842	1.75E-02	-0.019	8.95E-01
Mat ALT – CBMCs Density	0.842	1.75E-02	-0.019	8.95E-01
CBMCsConcentration-CBMCs Concentration	1.000	2.66E-15	0.993	<0.00001
CBHBsAg – CB AntiHBe	0.167	7.21E-01	0.292	3.37E-02
CBHBsAg – CBHBeAg	1.000	<0.00001	0.700	5.34E-09
CBHBeAg – CB AntiHBs	0.354	4.37E-01	0.287	3.75E-02
CBHBeAg – CB AntiHBe	0.167	7.21E-01	0.426	1.48E-03
CB AntiHBs – CB AntiHBe	0.471	2.86E-01	0.768	1.94E-11
Mat AntiHBs – CBMCs Concentration	0.965	4.19E-04	-0.050	7.23E-01
Mat AntiHBs – CBMCs Density	0.965	4.09E-04	-0.050	7.76E-01
Mat HBV DNA – Mat PBMCs Concetration	-0.803	2.98E-02	-0.290	3.94E-02
Mat HBV DNA – Mat PBMCs Density	-0.803	2.98E-02	-0.291	3.48E-02
Mat HBV DNA – CB HBsAg	-0.042	9.28E-01	0.394	3.52E-03
Mat HBV DNA – CB HBeAg	-0.042	9.28E-01	0.452	6.82E-04
Mat Creatinin – Mat PBMCs Concetration	0.185	6.92E-01	0.272	4.87E-02
Mat Creatinin – Mat PBMCs Density	0.185	6.92E-01	0.272	4.87E-02
Mat Albumin – Mat Blood Protein	0.999	1.57E-07	0.752	8.41E-11
Mat HBeAg – Mat HBV DNA	NA	NA	0.504	1.20E-04
Mat HBeAg – CBHBsAg	NA	NA	0.638	2.83E-07
Mat HBeAg – CBHBeAg	NA	NA	0.799	7.22E-13
Mat HBeAg – CBAntiHBe	NA	NA	0.367	6.86E-03
Mat PBMCs Concentration- Mat PBMCs Concentration	1.000	<0.00001	1.000	<0.00001
Mat PBMCs Concentration- CBMCs Concentration	-0.341	4.55E-01	0.473	3.43E-04
Mat PBMCs Concentration- CBMCs Density	-0.342	4.53E-01	0.449	7.36E-04
Mat PBMCs Density- CBMCs Concentration	-0.341	4.55E-01	0.474	3.41E-04
Mat PBMCs Density- CBMCs Density	-0.342	4.53E-01	0.450	7.33E-04

Table 4. Significant Correlation results in two group HBVDNA ≥ 5x10^{7}copies/ml (left) and HBVDNA < 5x10^7 copies/ml (right). Pair-wise Pearson correlation coefficients are shown in Supplementary table 1-4. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Taking the R values from the Pearson test (Supplementary Table 1 - 2) for the Fisher's Anova, we found the direction and power of correlation between Maternal Hemoglobin with other parameters significantly different two groups of viral load (p=3E-04). The same results were found with Maternal Prothrombin in Second (p=0.025) and Cord blood Anti HBs (p=0.025). (Supplementary Table 6)

Correlation and K-means change between two groups show the new cluster following HBV DNA concentration in mother serum

Base on the result of the correlation score (R) above, the clustering imputation show the diffrence in branching between the two groups of patients (figure 3). The agglomerative hierarchical clustering algorithms available in this program module build a cluster hierarchy that is commonly displayed as a tree diagram called a dendrogram. They begin with each object in a separate cluster. At each step, the two clusters that are most similar are joined into a single new cluster. Once fused, objects are never separated. Within each cluster, the value for this measure is displayed from smallest to largest. In the higher maternal viral load,

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Hemoglobin (MatHb) has the different behavior with other parameters. We do not find the same in the lower viral load group.

We used 20 different classifier clustering algorithms to determine and predict the number of clusters in two groups of patients (**Supplemetary table 7**). [17] Clustering is partitioning a set of objects into groups (clusters) so that entities within a group are more similar than objects in different groups. Most clustering algorithms depend on assumptions to define the subgroups present in a data set. Consequently, the resulting clustering scheme requires some evaluation regarding its validity. The evaluation procedure has to tackle difficult problems such as the quality of clusters, the degree with which a clustering scheme fits a specific data set and the optimal number of clusters in a partitioning. Using ANOVA test to compare the results of the clustering imputation following the 20 methods above in two viral load groups, we found the significant different results in the Ptbiserial, C-index, Mcclain and Sdindex method with p value are: 2.85E-02, 2.49E-02, 2.11E-06, 2.04E-02, respectively. **(Supplementary 7)** These methods proposed the differents optimal numbers of clusters.

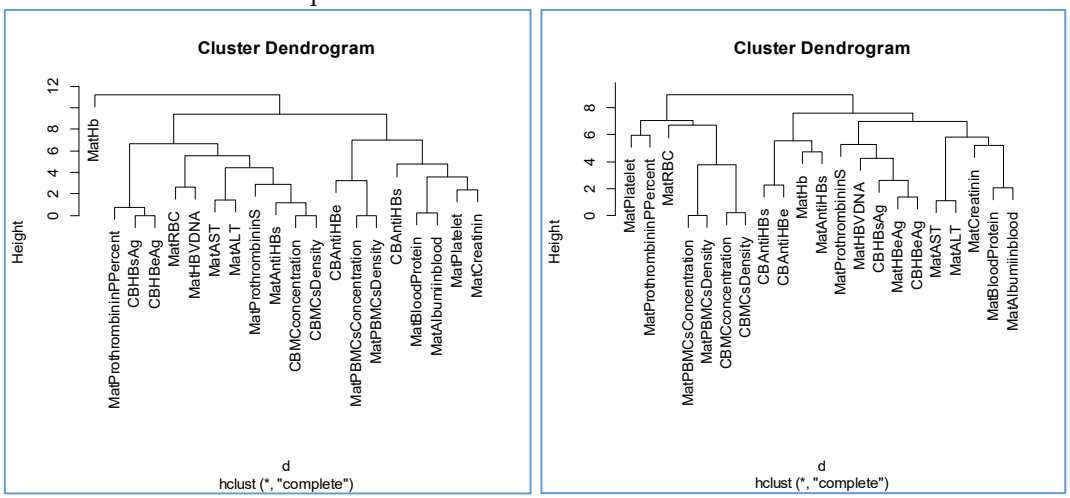

Figure 3. Cluster Dendrogram of two groups. (right) group of higher maternal viral load ([HBV DNA] ≥ **5x10**^{7} copies/ml), (left) group of lower maternal viral load ([HBV DNA] < **5x10**^{7}copies/ml). The height axis displays the Euclidean distance between observations or clusters. The horizontal bars indicate when two clusters/observations merged. HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

We chose the result proposed by the Ptbiserial method, in which the optimal cluster number for the higher viral load (groups HBVDNA ≥ 5x10^{7}copies/ml) is 5 and for the lower viral load (groups HBVDNA < 5x10^{7}copies/ml) is 9. The Ptbiserial index, examined by Milligan (1980, 1981) and Kraemer (1982), is simply a point-biserial correlation between the raw input dissimilarity matrix and a corresponding matrix consisting of 0 or 1 entries. A value of 0 is assigned if the two corresponding points are clustered together by the algorithm. A value of one is assigned otherwise (Milligan 1980). Given that larger positive values reflect a better fit between the data and the obtained partition, the maximum value

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of the index is used to select the optimal number of clusters in the data set (Milligan and Cooper 1985). The point biserial correlation coefficient is calculated using Equation 2 (Milligan 1981). [17]

$$Ptbiserial = \frac{[\bar{S}_b - \bar{S}_w][\frac{N_w N_b}{N_t^2}]^{1/2}}{S_d} \quad 17$$

Equation 2: The point biserial correlation coefficient. $\bar{S}_w = \frac{S_w}{N_w}$, $\bar{S}_b = \frac{S_b}{N_b}$, $S_d = standard\ deviation\ of\ all\ distances$, N_t = total number of pairs of observations in the data set $N_t = \frac{n(n-1)}{2}$. S_w = sum of the within-cluster distances $S_w = \sum_{k=1}^{q} \sum_{i,j \in C_k, i<j} d(x_i, x_j)$. N_w = total number of pairs of observations belonging to the same cluster $N_w = \sum_{k=1}^{q} \frac{n_k(n_k-1)}{2}$. N_b = total number of pairs of observations belonging to different clusters $N_b = N_t - N_w$. S_b = sum of the between-cluster distances $S_b = \sum_{k=1}^{q-1} \sum_{l=k+1}^{q} \sum_{i \in C_k, j \in C_l} d(x_i, x_j)$

The cluster plot in figure 4 described the differents distribution of intracluster variation between the two groups. For example, the CBMCs concentration and density locate in the same cluster with the maternal viral load (Mat HBV DNA) in the higher viral load group which is not the case in other group. (Figure 4) This result is also fit with the direction of association following Pearson's correlation method above (Figure 2). The objects located in the same cluster have the positif correlation.

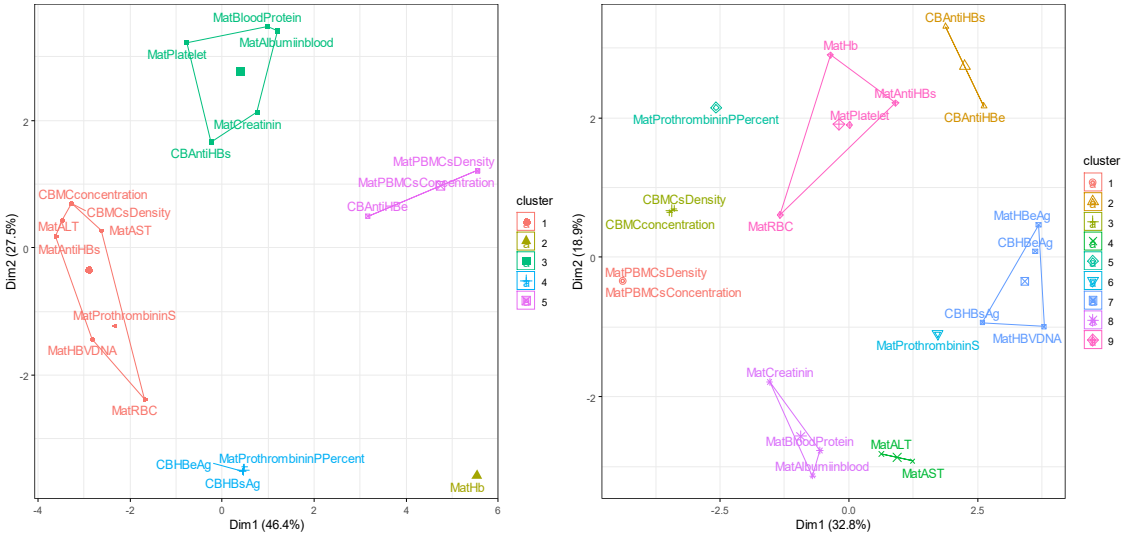

Figure 4: Cluster plot. (left) group of higher viral load ([HBV DNA] ≥ **5x10**^{7}copies/ml), (right) group of lower viral load ([HBV DNA] < **5x10**^{7}copies/ml). Cluster mapping between the parameters with cluster number = 5 and 9, respectively. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierarchical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

Discussion

New boundary index related to the presence of HBsAg in cord blood.

In our studies, among 60/80 pregnant CHB women, we identified 32 Cord blood HBsAg positive and 28 negative individuals. Taken the result of relative risk ratio test of Cord blood HBsAg positive probability, the boundary of maternal PBMCs concentration is 8.03E+06 cells/ml (with negative correlation) and CBMCs is 6.64E+06 cells/ml (with positive correlation). That means, HBsAg positive in blood may related with the increasing of CBMCs and the diminution of maternal PBMCs. Zhang et al (2005) showed that there were the significant different of the expression rate of CD80, CD83, IL12 between the healthy cord blood, the chronic hepatitis B mother cord blood and healthy cord blood. The T lymphocyte

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proliferation-inducing ability of dendritic cells of healthy adult peripheral blood was higher in inducing cord blood T lymphocytes proliferation.

In endemic areas, mother-to-child transmission (MTCT) is one of the primary sources of chronic HBV transmission.[18] Infants born to women who are positive for hepatitis B antigen (HBeAg) or have a high viral load (HBV DNA ≥ 10^6 copies/ml, or 2 × 10^5 IU/ml) are more prone to failure active-passive immunity prophylaxis. In addition, transmission could occur before (intrauterine), during, or after childbirth. Therefore, the failure of hepatitis B immune prophylaxis in infants delivered from HBV-infected mothers was assumed to be due to intrauterine infection[19]. Some research suggested that hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA appear in umbilical cord blood or peripheral blood of new-born children taken within a few days of birth might indicate intrauterine infection, leading to an MTCT rate of 30–60% [20–23]. This rate is much higher than the total HBsAg positive rate of 0.8–3.7% in infants delivered to HBV infected women following passive-active immuno-prophylaxis. [24–28]. After passive-active immuno-prophylaxis, nearly no children born to HBeAg-negative carrier women were infected with HBV, but chronic infection was still present in 4–12% of children born to HBeAg-positive mothers. [24–28]In babies delivered to HBeAg positive or highly viraemic (HBV DNA ≥ 10^6 copies/ml) moms who received antiviral medication during late pregnancy, the chronic HBV infection rate was almost negligible, according to more recent research.[29–31].

The role of peripheral blood mononuclear cells (PBMCs) in HBV intrauterine infection is still unclear. The origin of PBMCs in HBV-infected newborns, in particular, needs to be explored.[32] HBV immunoglobulin and vaccination can successfully block HBV MTCT during delivery post-partum in children of women positive for HBV surface antigen (HBsAg) to avoid viral transmission. However, for neonates with HBV intrauterine infection, there are two unresolved issues: passive-active immuno-prophylaxis failure and breakthrough HBV infections.[33–35]. As a result, efficient HBV infection management should prevent the virus from becoming transmitted during pregnancy. [33].

The presence of HBsAg and HBV DNA in newborn's serum is used to diagnose HBV intrauterine infection. Certain pregnant women have recently been reported to have HBV DNA and/or HBsAg in their peripheral blood mononuclear cells (PBMCs) but no HBsAg or HBV DNA in their serum.[36]. Their babies were infected with HBV and had severe hepatitis as a result [32]. Only the HBV DNA in neonatal PBMCs of certain HBV-infected newborn neonates was positive. These findings imply that HBV-infected maternal PBMC may play a key role in the transmission of HBV from mother to child, resulting in HBV infection in the womb. HBV DNA and/or HBsAg in PBMC might be useful indicators for HBV infection in newborns.[36] HBV-infected maternal PBMCs might reach the fetal bloodstream and induce HBV infection inside the womb. HBV may infect PBMCs and reproduce in them, according to reports. Infectious viral particles can also be released by HBV-infected PBMCs.[32,36] We also found 5x10^7 copies of HBV DNA/ml may become the new indication to define the probability of HBsAg positive in cord blood. When maternal viral load is from 5x10^7 copies/ml, the risk of being HBsAg positive in cord blood is 123%; when viral load is lower than this baseline, this risk is decreased by 55%. ($p<0.001$).

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Different clusters following HBV DNA concentration in mother serum

Method of K-means clustering by MacQueen (1967) is one of the most widely used unsupervised machine learning techniques for splitting a given data set into a collection of k groups. k is the number of groups that the analyst has pre-specified. It divides elements into numerous groups (clusters), with objects from the same cluster being as similar as possible (high intra-class similarity) and objects from other clusters being as dissimilar as possible (i.e., low inter-class similarity). Each cluster is represented by the center (i.e., centroid) in k-means clustering, which corresponds to the mean of points allocated to the cluster. The essential concept underlying k-means clustering is to define clusters so that total intra-cluster variance (also known as a total within-cluster variation) is minimized. The primary concept underlying k-means clustering is to define clusters with the least amount of intra-cluster variance (also known as total within-cluster variation). We want the overall within-cluster sum of squares to be as minimal as feasible since it measures the clustering's compactness (i.e., goodness).[37] Our study's goal is to find the closest index, especially when it comes to cord blood and maternal PBMCs, using clustering approaches. Cluster analysis revealed groupings that differed greatly from one another. The number of clusters must be determined ahead of time and submitted as a parameter to the k-means algorithm.[6]

We use 20 methods to describe to clustering of these subclinical parameters. They measures the quality of a clustering. It establishes how effectively each object is positioned inside its cluster. A good clustering is indicated by a high average silhouette width. For varying values of k, the average silhouette technique computes the average silhouette of observations. Over a range of feasible values for k, the ideal number of clusters k is the one that optimizes the average silhouette. In the higher viral load group, CBMCs concentration locates in the same cluster with serum maternal HBV DNA concentration. ALT, AST, AntiHBs, Prothrombin time, RBC. We show the deplacement of Maternal Prothrombin in Second (Mat Prothrombin in S) from cluster with maternal creatinin, maternal Protein in blood and maternal Albumin in blood in the lower viral load group to the cluster contain Maternal HBV DNA, CBMC concentrations in the higher viral load. The direction association change also from mild negative to significant positive, respectively. (Table 4)

We also found HBsAg in cord blood located in the same cluster with Maternal Prothrombin Percentage. That suggests that Prothrombin may be the critical factor that indirectly influences the evolution of MTCT probability. Besides that, other factors, such as Maternal Hb, Creatinin, Platelet, may also play an important role, because of their inversion correlation between two groups of viral load. (Table 2).

To our knowledge, the cluster analysis to identify distinct risk factors in a CHB pregnant women and their children (cord blood) has not been used previously. We show several results via our analyses of the clinically relevant features and maternal outcomes, leading to new insights about MTCT.

Conclusion

The substantial correlation shift of the subclinical score between two groups of viremic load might help detect chronic hepatitis B virus infection in pregnant women. This study looked at the characteristics of

Vietnamese CHB women during pregnancy. It shows to be a viable method for determining the heterogeneity of subclinical risk factors in CHB pregnant women. When comparing two groups of cord blood HBsAg positive and cord blood HBsAg negative, the relative risk ratio may help predict the risk of MTCT. Large-scale prospective studies with more pathology history, risk factors, and pregnancy problems are required for further investigation.

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"Apply DNA Sequencing and ELISPOT technologies in evaluation of HBV transmission risk from mother to children".

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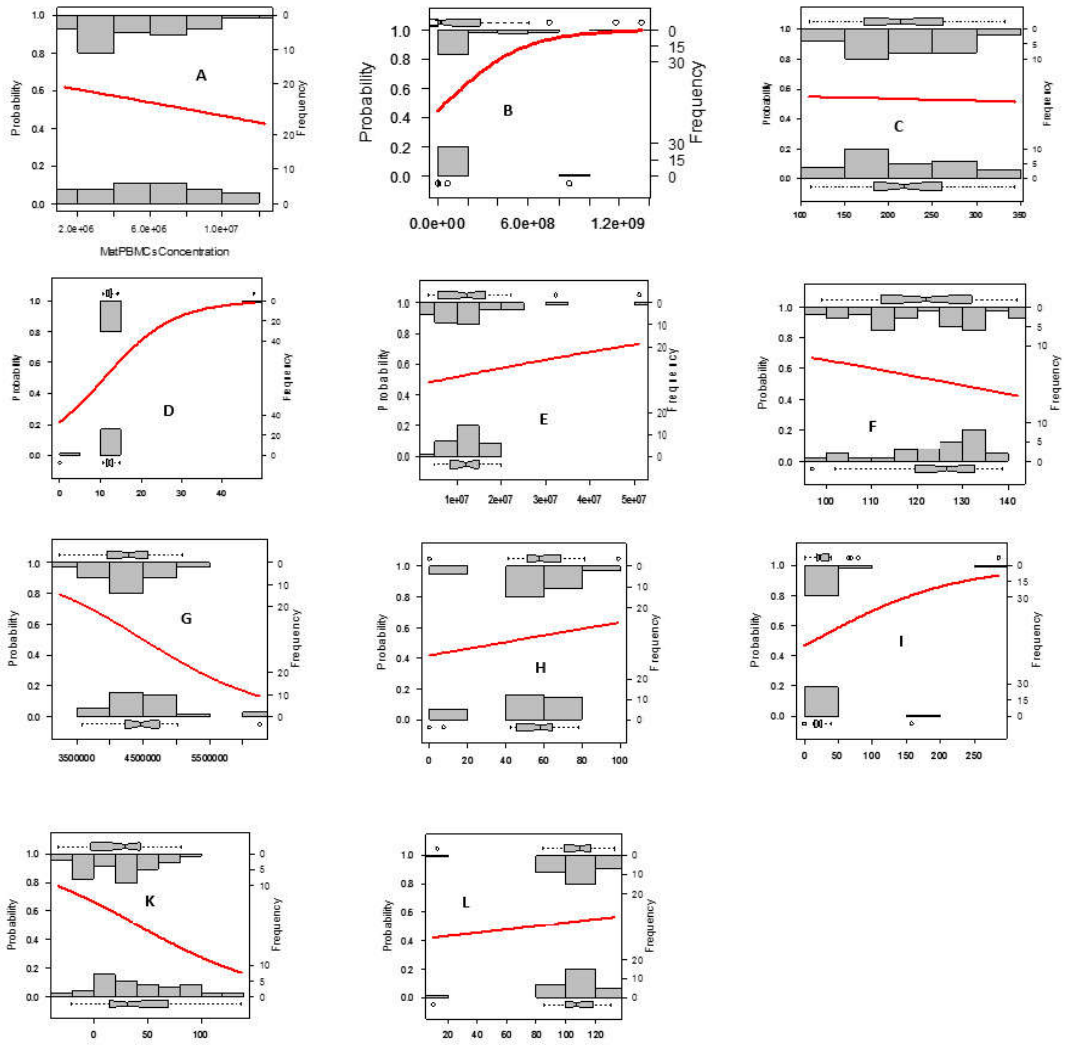

Supplementary figure 1: Relative Risk Ratio plot. Probability: 1: CBHBsAg positif, 0: CBHBsAg negatif: 0. More detail in table 1.

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	Mat RBC	Mat Hb	Mat Platelet	Mat ProthrombininS	Mat Prothrombinin PPercent	Mat AST	Mat ALT	Mat Creatinin	Mat Blood Protein	Mat Albumiin blood	Mat AntiHBs	Mat HBVDNA	Mat PBMCs Concentration	Mat PBMCs Density	CB HBsAg	CB HBeAg	CB AntiHBs	CB AntiHBe	CBMC concentration	CB MCsDensity
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.000	-0.205	0.044	0.360	-0.219	0.302	0.523	-0.233	-0.254	-0.283	0.594	0.779	-0.863	-0.863	-0.231	-0.231	-0.261	-0.300	0.379	0.380
2	-0.205	1.000	-0.412	-0.850	-0.226	-0.300	-0.533	-0.011	-0.568	-0.536	-0.779	-0.189	0.029	0.029	-0.318	-0.318	-0.766	-0.372	-0.880	-0.880
3	0.044	-0.412	1.000	0.103	-0.242	0.713	0.682	0.797	0.644	0.617	0.499	0.422	0.143	0.143	-0.305	-0.305	0.565	0.181	0.526	0.526
4	0.360	-0.850	0.103	1.000	0.563	0.341	0.525	-0.091	0.213	0.189	0.766	0.340	-0.386	-0.386	0.602	0.602	0.464	0.219	0.819	0.819
5	-0.219	-0.226	-0.242	0.563	1.000	0.228	0.095	0.003	-0.289	-0.288	0.119	0.025	-0.106	-0.106	0.969	0.969	0.259	0.208	0.225	0.225
6	0.302	-0.300	0.713	0.341	0.228	1.000	0.923	0.653	0.130	0.099	0.690	0.816	-0.411	-0.411	0.145	0.145	0.353	-0.228	0.646	0.646
7	0.523	-0.533	0.682	0.525	0.095	0.923	1.000	0.427	0.228	0.189	0.904	0.865	-0.530	-0.530	0.077	0.077	0.410	-0.263	0.842	0.842
8	-0.233	-0.011	0.797	-0.091	0.003	0.653	0.427	1.000	0.476	0.476	0.128	0.260	0.185	0.185	-0.165	-0.165	0.224	0.140	0.173	0.172
9	-0.254	-0.568	0.644	0.213	-0.289	0.130	0.228	0.476	1.000	0.999	0.320	-0.190	0.435	0.435	-0.267	-0.267	0.509	0.291	0.460	0.459
10	-0.283	-0.536	0.617	0.189	-0.288	0.099	0.189	0.476	0.999	1.000	0.281	-0.226	0.451	0.451	-0.270	-0.270	0.477	0.286	0.423	0.423
11	0.594	-0.779	0.499	0.766	0.119	0.690	0.904	0.128	0.320	0.281	1.000	0.719	-0.541	-0.541	0.167	0.167	0.471	-0.167	0.965	0.966
12	0.779	-0.189	0.422	0.340	0.025	0.816	0.865	0.260	-0.190	-0.226	0.719	1.000	-0.803	-0.803	-0.042	-0.042	-0.008	-0.395	0.546	0.547
13	-0.863	0.029	0.143	-0.386	-0.106	-0.411	-0.530	0.185	0.435	0.451	-0.541	-0.803	1.000	1.000	-0.067	-0.067	0.417	0.565	-0.341	-0.342
14	-0.863	0.029	0.143	-0.386	-0.106	-0.411	-0.530	0.185	0.435	0.451	-0.541	-0.803	1.000	1.000	-0.067	-0.067	0.417	0.565	-0.341	-0.342
15	-0.231	-0.318	-0.305	0.602	0.969	0.145	0.077	-0.165	-0.267	-0.270	0.167	-0.042	-0.067	-0.067	1.000	1.000	0.354	0.167	0.289	0.289
16	-0.231	-0.318	-0.305	0.602	0.969	0.145	0.077	-0.165	-0.267	-0.270	0.167	-0.042	-0.067	-0.067	1.000	1.000	0.354	0.167	0.289	0.289
17	-0.261	-0.766	0.565	0.464	0.259	0.353	0.410	0.224	0.509	0.477	0.471	-0.008	0.417	0.417	0.354	0.354	1.000	0.471	0.638	0.637
18	-0.300	-0.372	0.181	0.219	0.208	-0.228	-0.263	0.140	0.291	0.286	-0.167	-0.395	0.565	0.565	0.167	0.167	0.471	1.000	-0.031	-0.032
19	0.379	-0.880	0.526	0.819	0.225	0.646	0.842	0.173	0.460	0.423	0.965	0.546	-0.341	-0.341	0.289	0.289	0.638	-0.031	1.000	1.000
20	0.380	-0.880	0.526	0.819	0.225	0.646	0.842	0.172	0.459	0.423	0.966	0.547	-0.342	-0.342	0.289	0.289	0.637	-0.032	1.000	1.000

Supplementary table 1: R value from Pearson's correlation test in HBVDNA ≥ 5x10^{7}copies/ml group. The color-coded correlation factors between all the subclinical indexes including levels of Prothromibin, AST, ALT, RBC, Hb in mother blood; concentration and density of PBMCs, status of HbeAg, AntiHBs in Cord and Mother blood. The color value of the cells is proportional to the strength of the associations, ranging from red (negative correlations) to blue (positive correlations). The strength of the correlation is indicated in the color scale. Method: Pair-wise Pearson correlation coefficients. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

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	MatRBC	MatHb	MatPlatelet	MatProthrombininS	MatProthrombininPPercent	MatAST	MatALT	MatCreatinin	MatBloodProtein	MatAlbuminblood	MatHBeAg	MatAntiHBs	MatHBVDNA	MatPBMCsConcentration	MatPBMCsDensity	CBHBsAg	CBHBeAg	CBAntiHBs	CBAntiHBe	CBMCconcentration	CBMCsDensity
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1.000	0.199	-0.106	0.004	-0.073	0.064	0.070	-0.099	0.061	0.076	-0.197	-0.062	0.088	0.019	0.019	-0.305	-0.248	0.073	-0.006	-0.005	-0.002
2	0.199	1.000	0.113	0.048	0.046	-0.092	-0.018	-0.040	-0.049	-0.078	-0.045	0.257	0.063	0.066	0.066	-0.141	-0.069	0.254	0.177	-0.027	-0.044
3	-0.106	0.113	1.000	0.139	0.136	-0.100	-0.024	-0.070	0.035	-0.028	0.227	0.056	-0.022	-0.078	-0.078	-0.020	0.106	0.017	0.062	0.122	0.129
4	0.004	0.048	0.139	1.000	-0.745	0.051	0.054	0.148	0.227	0.235	0.221	0.158	0.238	-0.016	-0.016	0.177	0.223	0.071	0.054	-0.114	-0.115
5	-0.073	0.046	0.136	-0.745	1.000	-0.135	-0.084	-0.080	-0.002	-0.185	-0.113	0.008	-0.233	0.082	0.082	-0.065	-0.212	-0.174	-0.122	0.054	0.055
6	0.064	-0.092	-0.100	0.051	-0.135	1.000	0.896	0.155	0.119	0.152	0.136	-0.039	0.605	-0.088	-0.088	0.129	0.177	-0.167	-0.046	-0.030	-0.030
7	0.070	-0.018	-0.024	0.054	-0.084	0.896	1.000	0.153	0.193	0.208	0.050	-0.019	0.430	-0.015	-0.015	0.044	0.090	-0.173	-0.042	-0.019	-0.019
8	-0.099	-0.040	-0.070	0.148	-0.080	0.155	0.153	1.000	0.233	0.197	0.010	0.155	0.002	0.272	0.272	0.139	0.006	-0.182	0.027	0.209	0.204
9	0.061	-0.049	0.035	0.227	-0.002	0.119	0.193	0.233	1.000	0.752	-0.001	0.065	0.060	0.156	0.156	0.115	0.050	-0.089	-0.055	-0.105	-0.116
10	0.076	-0.078	-0.028	0.235	-0.185	0.152	0.208	0.197	0.752	1.000	-0.025	-0.159	0.172	0.197	0.196	0.172	0.088	-0.066	0.058	0.114	0.106
11	-0.197	-0.045	0.227	0.221	-0.113	0.136	0.050	0.010	-0.001	-0.025	1.000	0.110	0.504	-0.259	-0.258	0.638	0.799	0.170	0.367	-0.037	-0.023
12	-0.062	0.257	0.056	0.158	0.008	-0.039	-0.019	0.155	0.065	-0.159	0.110	1.000	0.040	-0.140	-0.140	-0.032	0.095	0.253	0.162	-0.050	-0.040
13	0.088	0.063	-0.022	0.238	-0.233	0.605	0.430	0.002	0.060	0.172	0.504	0.040	1.000	-0.290	-0.291	0.394	0.452	0.270	0.441	-0.179	-0.172
14	0.019	0.066	-0.078	-0.016	0.082	-0.088	-0.015	0.272	0.156	0.197	-0.259	-0.140	-0.290	1.000	1.000	-0.068	-0.190	-0.096	-0.048	0.473	0.449
15	0.019	0.066	-0.078	-0.016	0.082	-0.088	-0.015	0.272	0.156	0.196	-0.258	-0.140	-0.291	1.000	1.000	-0.069	-0.190	-0.096	-0.048	0.474	0.450
16	-0.305	-0.141	-0.020	0.177	-0.065	0.129	0.044	0.139	0.115	0.172	0.638	-0.032	0.394	-0.068	-0.069	1.000	0.700	-0.041	0.292	0.004	-0.012
17	-0.248	-0.069	0.106	0.223	-0.212	0.177	0.090	0.006	0.050	0.088	0.799	0.095	0.452	-0.190	-0.190	0.700	1.000	0.287	0.426	0.035	0.047
18	0.073	0.254	0.017	0.071	-0.174	-0.167	-0.173	-0.182	-0.089	-0.066	0.170	0.253	0.270	-0.096	-0.096	-0.041	0.287	1.000	0.768	0.014	0.029
19	-0.006	0.177	0.062	0.054	-0.122	-0.046	-0.042	0.027	-0.055	0.058	0.367	0.162	0.441	-0.048	-0.048	0.292	0.426	0.768	1.000	-0.046	-0.041
20	-0.005	-0.027	0.122	-0.114	0.054	-0.030	-0.019	0.209	-0.105	0.114	-0.037	-0.050	-0.179	0.473	0.474	0.004	0.035	0.014	-0.046	1.000	0.993
21	-0.002	-0.044	0.129	-0.115	0.055	-0.030	-0.019	0.204	-0.116	0.106	-0.023	-0.040	-0.172	0.449	0.450	-0.012	0.047	0.029	-0.041	0.993	1.000

Supplementary table 2: R value from Pearson's correlation test in HBVDNA < 5x10^{7}copies/ml group. The color-coded correlation factors between all the subclinical indexes including levels of Prothromibin, AST, ALT, RBC, Hb in mother blood; concentration and density of PBMCs, status of HbeAg, AntiHBs in Cord and Mother blood. The color value of the cells is proportional to the strength of the associations, ranging from red (negative correlations) to blue (positive correlations). The strength of the correlation is indicated in the color scale. Method: Pair-wise Pearson correlation coefficients. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity.

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	MatRBC	MatHb	MatPlatelet	MatProthrombininS	MatProthrombininPPercent	MatAST	MatALT	MatCreatinin	MatBloodProtein	MatAlbuminblood	MatAntiHBs	MatHBVDNA	MatPBMCsConcentration	MatPBMCsDensity	CBHBsAg	CBHBeAg	CBAntiHBs	CBAntiHBe	CBMCconcentration	CBMCsDensity
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	NA	6.59E-01	9.25E-01	4.27E-01	6.37E-01	5.10E-01	2.29E-01	6.14E-01	5.83E-01	5.39E-01	1.60E-01	3.89E-02	1.24E-02	1.24E-02	6.18E-01	6.18E-01	5.72E-01	5.13E-01	4.02E-01	4.00E-01
2	6.59E-01	NA	3.58E-01	1.55E-02	6.27E-01	5.14E-01	2.18E-01	9.81E-01	1.83E-01	2.15E-01	3.89E-02	6.85E-01	9.50E-01	9.50E-01	4.87E-01	4.87E-01	4.44E-02	4.12E-01	8.97E-03	9.05E-03
3	9.25E-01	3.58E-01	NA	8.26E-01	6.01E-01	7.24E-02	9.14E-02	3.18E-02	1.18E-01	1.40E-01	2.54E-01	3.46E-01	7.60E-01	7.60E-01	5.07E-01	5.07E-01	1.87E-01	6.98E-01	2.25E-01	2.26E-01
4	4.27E-01	1.55E-02	8.26E-01	NA	1.88E-01	4.54E-01	2.27E-01	8.46E-01	6.46E-01	6.85E-01	4.48E-02	4.56E-01	3.93E-01	3.93E-01	1.53E-01	1.53E-01	2.94E-01	6.37E-01	2.41E-02	2.41E-02
5	6.37E-01	6.27E-01	6.01E-01	1.88E-01	NA	6.23E-01	8.39E-01	9.94E-01	5.30E-01	5.31E-01	8.00E-01	9.57E-01	8.22E-01	8.22E-01	3.19E-04	3.19E-04	5.74E-01	6.54E-01	6.27E-01	6.27E-01
6	5.10E-01	5.14E-01	7.24E-02	4.54E-01	6.23E-01	NA	3.03E-03	1.12E-01	7.82E-01	8.33E-01	8.60E-02	2.51E-02	3.60E-01	3.60E-01	7.56E-01	7.56E-01	4.37E-01	6.23E-01	1.17E-01	1.17E-01
7	2.29E-01	2.18E-01	9.14E-02	2.27E-01	8.39E-01	3.03E-03	NA	3.39E-01	6.22E-01	6.85E-01	5.18E-03	1.19E-02	2.21E-01	2.21E-01	8.70E-01	8.70E-01	3.61E-01	5.69E-01	1.75E-02	1.74E-02
8	6.14E-01	9.81E-01	3.18E-02	8.46E-01	9.94E-01	1.12E-01	3.39E-01	NA	2.80E-01	2.80E-01	7.85E-01	5.74E-01	6.92E-01	6.92E-01	7.24E-01	7.24E-01	6.29E-01	7.64E-01	7.11E-01	7.12E-01
9	5.83E-01	1.83E-01	1.18E-01	6.46E-01	5.30E-01	7.82E-01	6.22E-01	2.80E-01	NA	1.57E-07	4.83E-01	6.83E-01	3.29E-01	3.29E-01	5.63E-01	5.63E-01	2.43E-01	5.27E-01	3.00E-01	3.00E-01
10	5.39E-01	2.15E-01	1.40E-01	6.85E-01	5.31E-01	8.33E-01	6.85E-01	2.80E-01	1.57E-07	NA	5.42E-01	6.27E-01	3.09E-01	3.09E-01	5.58E-01	5.58E-01	2.79E-01	5.34E-01	3.44E-01	3.45E-01
11	1.60E-01	3.89E-02	2.54E-01	4.48E-02	8.00E-01	8.60E-02	5.18E-03	7.85E-01	4.83E-01	5.42E-01	NA	6.88E-02	2.10E-01	2.10E-01	7.21E-01	7.21E-01	2.86E-01	7.21E-01	4.19E-04	4.09E-04
12	3.89E-02	6.85E-01	3.46E-01	4.56E-01	9.57E-01	2.51E-02	1.19E-02	5.74E-01	6.83E-01	6.27E-01	6.88E-02	NA	2.98E-02	2.98E-02	9.28E-01	9.28E-01	9.86E-01	3.80E-01	2.05E-01	2.04E-01
13	1.24E-02	9.50E-01	7.60E-01	3.93E-01	8.22E-01	3.60E-01	2.21E-01	6.92E-01	3.29E-01	3.09E-01	2.10E-01	2.98E-02	NA	0.00E+00	8.87E-01	8.87E-01	3.52E-01	1.86E-01	4.55E-01	4.53E-01
14	1.24E-02	9.50E-01	7.60E-01	3.93E-01	8.22E-01	3.60E-01	2.21E-01	6.92E-01	3.29E-01	3.09E-01	2.10E-01	2.98E-02	0.00E+00	NA	8.87E-01	8.87E-01	3.52E-01	1.86E-01	4.55E-01	4.53E-01
15	6.18E-01	4.87E-01	5.07E-01	1.53E-01	3.19E-04	7.56E-01	8.70E-01	7.24E-01	5.63E-01	5.58E-01	7.21E-01	9.28E-01	8.87E-01	8.87E-01	NA	0.00E+00	4.37E-01	7.21E-01	5.29E-01	5.30E-01
16	6.18E-01	4.87E-01	5.07E-01	1.53E-01	3.19E-04	7.56E-01	8.70E-01	7.24E-01	5.63E-01	5.58E-01	7.21E-01	9.28E-01	8.87E-01	8.87E-01	0.00E+00	NA	4.37E-01	7.21E-01	5.29E-01	5.30E-01
17	5.72E-01	4.44E-02	1.87E-01	2.94E-01	5.74E-01	4.37E-01	3.61E-01	6.29E-01	2.43E-01	2.79E-01	2.86E-01	9.86E-01	3.52E-01	3.52E-01	4.37E-01	4.37E-01	NA	2.86E-01	1.23E-01	1.24E-01
18	5.13E-01	4.12E-01	6.98E-01	6.37E-01	6.54E-01	6.23E-01	5.69E-01	7.64E-01	5.27E-01	5.34E-01	7.21E-01	3.80E-01	1.86E-01	1.86E-01	7.21E-01	7.21E-01	2.86E-01	NA	9.47E-01	9.45E-01
19	4.02E-01	8.97E-03	2.25E-01	2.41E-02	6.27E-01	1.17E-01	1.75E-02	7.11E-01	3.00E-01	3.44E-01	4.19E-04	2.05E-01	4.55E-01	4.55E-01	5.29E-01	5.29E-01	1.23E-01	9.47E-01	NA	2.66E-15
20	4.00E-01	9.05E-03	2.26E-01	2.41E-02	6.27E-01	1.17E-01	1.74E-02	7.12E-01	3.00E-01	3.45E-01	4.09E-04	2.04E-01	4.53E-01	4.53E-01	5.30E-01	5.30E-01	1.24E-01	9.45E-01	2.66E-15	NA

Supplementary table 3: p value from Pearson's correlation test in HBVDNA ≥ 5x10^{7}copies/ml group. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	NA	1.52E-01	4.50E-01	9.80E-01	6.02E-01	6.51E-01	6.17E-01	4.80E-01	6.64E-01	5.89E-01	1.57E-01	6.57E-01	5.29E-01	8.94E-01	8.91E-01	2.66E-02	7.38E-02	6.05E-01	9.69E-01	9.70E-01	9.87E-01
2	1.52E-01	NA	4.20E-01	7.35E-01	7.42E-01	5.14E-01	8.99E-01	7.74E-01	7.26E-01	5.78E-01	7.48E-01	6.36E-02	6.52E-01	6.41E-01	6.40E-01	3.13E-01	6.25E-01	6.65E-02	2.04E-01	8.46E-01	7.56E-01
3	4.50E-01	4.20E-01	NA	3.20E-01	3.32E-01	4.75E-01	8.66E-01	6.20E-01	8.01E-01	8.42E-01	1.02E-01	6.89E-01	8.74E-01	5.80E-01	5.80E-01	8.86E-01	4.50E-01	9.05E-01	6.61E-01	3.85E-01	3.59E-01
4	9.80E-01	7.35E-01	3.20E-01	NA	1.52E-10	7.19E-01	7.01E-01	2.89E-01	1.02E-01	9.04E-02	1.12E-01	2.58E-01	8.68E-02	9.07E-01	9.07E-01	2.04E-01	1.08E-01	6.15E-01	7.01E-01	4.15E-01	4.13E-01
5	6.02E-01	7.42E-01	3.32E-01	1.52E-10	NA	3.34E-01	5.51E-01	5.68E-01	9.88E-01	1.84E-01	4.21E-01	9.57E-01	9.25E-02	5.61E-01	5.59E-01	6.41E-01	1.28E-01	2.12E-01	3.86E-01	6.99E-01	6.96E-01
6	6.51E-01	5.14E-01	4.75E-01	7.19E-01	3.34E-01	NA	0.00E+00	2.68E-01	3.94E-01	2.76E-01	3.33E-01	7.80E-01	1.61E-06	5.31E-01	5.30E-01	3.56E-01	2.05E-01	2.32E-01	7.42E-01	8.29E-01	8.30E-01
7	6.17E-01	8.99E-01	8.66E-01	7.01E-01	5.51E-01	0.00E+00	NA	2.75E-01	1.66E-01	1.35E-01	7.23E-01	8.92E-01	1.29E-03	9.12E-01	9.12E-01	7.54E-01	5.23E-01	2.16E-01	7.65E-01	8.95E-01	8.95E-01
8	4.80E-01	7.74E-01	6.20E-01	2.89E-01	5.68E-01	2.68E-01	2.75E-01	NA	9.25E-02	1.58E-01	9.44E-01	2.66E-01	9.86E-01	4.87E-02	4.85E-02	3.21E-01	9.67E-01	1.92E-01	8.47E-01	1.34E-01	1.43E-01
9	6.64E-01	7.26E-01	8.01E-01	1.02E-01	9.88E-01	3.94E-01	1.66E-01	9.25E-02	NA	8.41E-11	9.96E-01	6.44E-01	6.67E-01	2.64E-01	2.65E-01	4.13E-01	7.22E-01	5.28E-01	6.93E-01	4.55E-01	4.08E-01
10	5.89E-01	5.78E-01	8.42E-01	9.04E-02	1.84E-01	2.76E-01	1.35E-01	1.58E-01	8.41E-11	NA	8.57E-01	2.57E-01	2.17E-01	1.58E-01	1.59E-01	2.17E-01	5.33E-01	6.36E-01	6.83E-01	4.17E-01	4.52E-01
11	1.57E-01	7.48E-01	1.02E-01	1.12E-01	4.21E-01	3.33E-01	7.23E-01	9.44E-01	9.96E-01	8.57E-01	NA	4.32E-01	1.20E-04	6.15E-02	6.17E-02	2.83E-07	7.22E-13	2.24E-01	6.86E-03	7.95E-01	8.69E-01
12	6.57E-01	6.36E-02	6.89E-01	2.58E-01	9.57E-01	7.80E-01	8.92E-01	2.66E-01	6.44E-01	2.57E-01	4.32E-01	NA	7.74E-01	3.17E-01	3.17E-01	8.18E-01	4.99E-01	6.72E-02	2.45E-01	7.23E-01	7.76E-01
13	5.29E-01	6.52E-01	8.74E-01	8.68E-02	9.25E-02	1.61E-06	1.29E-03	9.86E-01	6.67E-01	2.17E-01	1.20E-04	7.74E-01	NA	3.49E-02	3.48E-02	3.52E-03	6.82E-04	5.05E-02	9.41E-04	1.99E-01	2.17E-01
14	8.94E-01	6.41E-01	5.80E-01	9.07E-01	5.61E-01	5.31E-01	9.12E-01	4.87E-02	2.64E-01	1.58E-01	6.15E-02	3.17E-01	3.49E-02	NA	0.00E+00	6.27E-01	1.74E-01	4.94E-01	7.32E-01	3.43E-04	7.36E-04
15	8.91E-01	6.40E-01	5.80E-01	9.07E-01	5.59E-01	5.30E-01	9.12E-01	4.85E-02	2.65E-01	1.59E-01	6.17E-02	3.17E-01	3.48E-02	0.00E+00	NA	6.26E-01	1.73E-01	4.93E-01	7.32E-01	3.41E-04	7.33E-04
16	2.66E-02	3.13E-01	8.86E-01	2.04E-01	6.41E-01	3.56E-01	7.54E-01	3.21E-01	4.13E-01	2.17E-01	2.83E-07	8.18E-01	3.52E-03	6.27E-01	6.26E-01	NA	5.34E-09	7.73E-01	3.37E-02	9.76E-01	9.31E-01
17	7.38E-02	6.25E-01	4.50E-01	1.08E-01	1.28E-01	2.05E-01	5.23E-01	9.67E-01	7.22E-01	5.33E-01	7.22E-13	4.99E-01	6.82E-04	1.74E-01	1.73E-01	5.34E-09	NA	3.75E-02	1.48E-03	8.05E-01	7.40E-01
18	6.05E-01	6.65E-02	9.05E-01	6.15E-01	2.12E-01	2.32E-01	2.16E-01	1.92E-01	5.28E-01	6.36E-01	2.24E-01	6.72E-02	5.05E-02	4.94E-01	4.93E-01	7.73E-01	3.75E-02	NA	1.94E-11	9.20E-01	8.36E-01
19	9.69E-01	2.04E-01	6.61E-01	7.01E-01	3.86E-01	7.42E-01	7.65E-01	8.47E-01	6.93E-01	6.83E-01	6.86E-03	2.45E-01	9.41E-04	7.32E-01	7.32E-01	3.37E-02	1.48E-03	1.94E-11	NA	7.43E-01	7.71E-01
20	9.70E-01	8.46E-01	3.85E-01	4.15E-01	6.99E-01	8.29E-01	8.95E-01	1.34E-01	4.55E-01	4.17E-01	7.95E-01	7.23E-01	1.99E-01	3.43E-04	3.41E-04	9.76E-01	8.05E-01	9.20E-01	7.43E-01	NA	0.00E+00
21	9.87E-01	7.56E-01	3.59E-01	4.13E-01	6.96E-01	8.30E-01	8.95E-01	1.43E-01	4.08E-01	4.52E-01	8.69E-01	7.76E-01	2.17E-01	7.36E-04	7.33E-04	9.31E-01	7.40E-01	8.36E-01	7.71E-01	0.00E+00	NA

Supplementary table 4: p value from Pearson's correlation test in HBVDNA < 5x10^{7}copies/ml group. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	F value	**Pr(>F)**	Signification code

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MatRBC	1.3	0.26	
MatHb	0.29	0.59	
MatPlatelet	0.41	0.52	
MatProthrombininS	0.11	0.73	
MatProthrombininPPercent	1.42	0.24	
MatAST	0.24	0.63	
MatALT	1.65	0.20	
MatCreatinin	0.07	0.80	
MatBloodProtein	0.37	0.55	
MatAlbumiinblood	0.14	0.70	
MatHBeAg		na	
MatAntiHBs	1.33	0.25	
MatPBMCsConcentration	0.02	0.89	
MatPBMCsDensity	0.02	0.89	
CBHBsAg	3.41	0.07	.
CBAntiHBs	0.70	0.41	
CBAntiHBe	8.32	0.006	**
CBMCconcentration	1.10	0.30	
CBMCsDensity	1.17	0.28	

Supplementary table 5: ANOVA test between two groups, HBVDNA < $5x10^7$ copies/ml and HBVDNA ≥ $5x10^7$copies/ml. Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Mat RBC	1	0	0.0004	0.003	0.96	
Mat Hb	**1**	**1.9336**	**1.93358**	**15.961**	**0.000278**	***
Mat Platelet	**1**	**0.5905**	**0.59046**	**5.4034**	**0.0254**	*
Mat Prothrombin in S	1	0.4671	0.4671	3.1307	0.08465	.
Mat Prothrombin P Percent	1	0.4392	0.43917	3.3942	0.07304	.
Mat AST	1	0.3977	0.39766	2.7468	0.1055	
Mat ALT	1	0.4354	0.4354	2.5212	0.1204	
Mat Creatinin	1	0.1077	0.10771	1.3257	0.2566	
Mat Blood Protein	1	0.112	0.11203	0.8776	0.3546	
Mat Albumin in blood	1	0.0546	0.054602	0.4284	0.5166	
Mat AntiHBs	1	0.6738	0.67381	4.0511	0.05108	.
Mat PBMCs Concentration	1	0.165	0.16501	0.8041	0.3754	
Mat PBMCs Density	1	0.1651	0.16508	0.8043	0.3753	
CB AntiHBsAg	1	0.0045	0.004524	0.0322	0.8586	
CB AntiHBs	1	0.6311	0.63114	5.6501	0.02246	*
CB AntiHBe	1	0.0143	0.014309	0.1329	0.7174	
CBMCs Concentration	1	0.6116	0.61159	3.5226	0.06803	.
CBMCs Density	1	0.6183	0.61833	3.5785	0.06597	.

Supplementary table 6: **p value Fisher test and Pearson's Test between the R from two groups, HBVDNA < 5x10^{7}copies/ml and HBVDNA ≥ 5x10^{7}copies/ml.** Abbreviations: HBV, hepatitis B virus; PBMCs, Peripheral Blood Mononuclear Cells; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; Hb, Hemoglobin; RBC, Red Blood Cell; CBMC, umbilical cord blood mononuclear cells, Mat: Mother or Maternal, CB: Cord blood, HCA: Hierachical cluster analysis, ProthrfombininS: Prothrombin time in second, ProthrombininPercent: Prothrombin % activity. Pair-wise Pearson correlation coefficients are shown in Supplementary table 1-4. Abbreviations: **Df**, The degrees of freedom; **Sum Sq**, the Sum of squares, helps to express the total variation that can be attributed to various factors; **Mean Sq**, the Mean squares, are used to determine whether factors (treatments) are significant; **Pr(>F)**, the p-value associated with the F statistic (**F value**) of a given effect and test statistic. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Index	P value	F value			Sum square on site		Df on site	Df on Residuals	HBV DNA ≥ 5*10^5 copies/ml	HBV DNA < 5*10^5 copies/ml

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			Mean square on site	Mean square on Residuals		Sum square on Residuals			Number cluster optimal	Value Index	Number cluster optimal	Value Index
1) Kl	3.11E-01	1.06	2.20E+52	2.08E+52	2.20E+52	6.85E+53	1	33	NA	NA	19	38.86
2) Ch	3.11E-01	1.06	9.69E+64	9.14E+64	9.69E+64	3.02E+66	1	33	NA	NA	19	3039.51
3) Hartigan	2.96E-01	1.13	2.19E+53	1.94E+53	2.19E+53	6.19E+54	1	32	NA	NA	19	4737.30
4) C-index	2.49E-02	5.51	2.74E-01	4.97E-02	2.74E-01	1.69E+00	1	34	19	0.00	19	0.03
5) Db	8.02E-02	3.25	5.78E-01	1.78E-01	5.78E-01	6.04E+00	1	34	19	0.00	19	0.01
6) Duda	3.11E-01	1.06	1.32E+65	1.25E+65	1.32E+65	4.11E+66	1	33	2	0.71	2	0.77
7) Pseudot2	6.52E-01	0.21	3.27E+00	1.58E+01	3.27E+00	5.39E+02	1	34	2	2.83	2	3.93
8) Ratkowsky	1.45E-01	2.23	5.94E-03	2.67E-03	5.94E-03	9.08E-02	1	34	3	0.41	3	0.35
9) Ball	6.02E-01	0.28	7.28E+00	2.62E+01	7.28E+00	8.92E+02	1	34	3	11.95	3	6.22
10) Ptbiserial	**2.85E-02**	**5.23**	**6.95E-02**	**1.33E-02**	**6.95E-02**	**4.52E-01**	**1**	**34**	**5**	**0.69**	**9**	**0.65**
11) Mcclain	2.11E-06	32.50	6.19E+01	1.91E+00	6.19E+01	6.48E+01	1	34	19	0.00	2	0.57
12) Gamma	8.88E-01	0.02	3.08E-04	1.54E-02	3.08E-04	5.24E-01	1	34	13	1.00	14	1.00
13) Gplus	9.53E-01	0.00	2.97E-02	8.52E+00	2.97E-02	2.90E+02	1	34	13	0.00	14	0.00
14) Tau	7.92E-01	0.07	8.11E+00	1.15E+02	8.11E+00	3.91E+03	1	34	3	32.65	2	34.30
15) Dunn	3.11E-01	1.06	3.68E+25	3.47E+25	3.68E+25	1.15E+27	1	33	NA	NA	19	6.25
16) Sdindex	2.04E-02	5.92	1.25E+32	2.11E+31	1.25E+32	7.18E+32	1	34	18	718.37	14	1.55
17) Sdbw	5.72E-02	3.88	2.25E-01	5.80E-02	2.25E-01	1.97E+00	1	34	19	0.00	19	0.00
18) Elbow kmeans	3.28E-01	1.10	3.48E+02	3.44E+02	3.48E+02	6.20E+03	1	18	NA	NA	NA	NA
19) Silhouette kmeans	2.03E-01	1.74	3.29E-02	1.89E-02	3.29E-02	3.39E-01	1	18	5	0.28	8	0.26
20) Gap Statistic kmeans	3.02E-01	1.13	4.23E-02	3.74E-02	4.23E-02	6.74E-01	1	18	10	0.69	10	0.40
21) Gap Statistic hierachical clustering	2.72E-01	1.29	4.77E-02	3.70E-02	4.77E-02	6.66E-01	1	18	10	0.70	10	0.40

Supplementary table 7: **Results of clustering imputation following three methods (Elbow, Silhouette and Gap statistic) for two groups, HBVDNA < 5x10^{7}copies/ml and HBVDNA ≥ 5x10^{7}copies/ml.** Abbreviations: CH (Calinski and Harabasz 1974), CCC (Sarle 1983), Pseudot2 (Duda and Hart 1973), KL (Krzanowski and Lai 1988), Gamma (Baker and Hubert 1975), Gap (Tibshirani et al. 2001), Silhouette (Rousseeuw 1987), Hartigan (Hartigan 1975), Cindex (Hubert and Levin 1976), DB (Davies and Bouldin 1979), Ratkowsky (Ratkowsky and Lance 1978), Scott (Scott and Symons 1971), Marriot (Marriot 1971), Ball (Ball and Hall 1965), Trcovw (Milligan and Cooper 1985), Tracew (Milligan and Cooper 1985), Friedman (Friedman and Rubin 1967), Rubin (Friedman and Rubin 1967), Dunn (Dunn 1974). **Df**, The degrees of freedom; **Sum Sq**, the Sum of squares, helps to express the total variation that can be attributed to various factors; **Mean Sq**, the Mean squares, are used to determine whether factors (treatments) are significant; **Pr(>F)**, the p-value associated with the F statistic (**F value**) of a given effect and test statistic. Significant statistic: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1