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Expression of Natural Killer Cell Inhibitory Receptors is Associated with Significant Liver Injury in Chronic Hepatitis C in Children

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ABSTRACT

Introduction and aim. Natural Killer (NK) cells play an important role in innate immune response to viral infections and their high proportion is situated in the liver. The aim of this study was to analyze possible relation between the expression of NK cell receptors and varied intensity of liver lesions in chronic hepatitis C (CHC) in children. **Material and methods.** Study included 105 children with CHC - 54 boys and 51 girls, age 13.62 ± 3.48 years. Blood specimens were taken at the day of the liver biopsy. Histological evaluation was performed according to METAVIR scoring system. Circulating NK cells were evaluated by flow cytometry. The results were shown as a proportion of cells expressing evaluated receptor and its' mean fluorescent intensity (MFI). **Results.** In 58 children with CHC (55.2%) significant liver fibrosis was observed (≥F2). Higher proportion of cells expressing CD158e inhibitory receptors was observed in the group of children with ALT > 2UNL (21.11 ± 14.60 *vs.* 12.22 ± 8.99%; p = 0.037). While higher proportion of cells expressing inhibitory CD158b receptor was observed in children with significant fibrosis (F ≥ 2) compared to minimal fibrosis (F < 2) - (34.14 ± 12.44 vs. 27.48 ± 8.71%; p = 0.049). Children with advanced fibrosis (F ≥ 3) had higher MFI of NK cell CD 158b receptor than children with fibrosis scored F < 3 - (5344.20 ± 3407.49 *vs.* 2979.67 ± 1190.64; p = 0.049). Proportion of NK cells expressing CD158b was found a predictor of significant fibrosis in univariate analysis - [OR 1.065; 95%CI (1.07-1.15); p = 0.046]. **Conclusions.** Higher proportion of NK cells expressing inhibitory CD158b and CD158e receptors is associated with significant liver injury.

Key words. Liver inflammation. Fibrosis. Steatosis. Innate immunity.

INTRODUCTION

Chronic hepatitis C (CHC) is considered to have a relatively slowly progressing clinical course in children, giving mild to moderate liver lesions.[1] Nevertheless, some patients may develop serious complications in childhood including liver cirrhosis.[2] Determination of such patients is extremely important for a proper clinical management. Therefore, a search for the risk factors of the aggravation of the clinical course seems to enable the determination of patients that are at greatest risk to develop significant complications as liver cirrhosis.[3]

Natural killer (NK) cells play an important role in innate immune response in the course of viral infection as important anti-viral effectors. Their high proportion is situated in the liver, where they respond to a large number of antigens that are transported to this organ from the gastrointestinal tract.[4] They provide anti-viral protection through direct killing of infected cells or the production of immunoregulatory cytokines influencing adaptive immune response. NK cells express a large number of receptors on their surface, which activation leads to inhibition or stimulation of their killing potential.[5] Numerous families of NK cell receptors were described so far. Three most important groups include: natural cytotoxicity receptors, killer cells immunoglobulin-like receptors (KIR), lectin-like receptors type C.[6] Various ligands bind to the receptors: viral particles, MHC class I proteins, other proteins and glicosaminoglicans. KIR receptors are marked as CD158 particles with subsequent letters a-k.[7]

In chronic HCV infection, NK cells have been shown to kill infected hepatocytes. Furthermore, they may express varied phenotype and function. In general, data concerning the number of circulating NK cells in CHC are conflicting.

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Golden-Mason, *et al*. reported decreased number of NK cells compared to healthy individuals in the study conducted on the group of HCV infected women.[8] Varchetta, *et al.* mentioned altered cytotoxic function of these cells in CHC.[9] Furthermore, Ahlenstiel, et al. observed that patients with normal ALT activity had normal NK cell function, while those with higher ALT levels displayed higher cytotoxicity against target cells.[10] Moreover, activated NK cell phenotypes were linked to the spontaneous HCV clearance, while the expression of inhibitory receptors was related to poorer treatment outcome.[8,11] NK cells are considered to have inhibitory potential regarding liver fibrosis. Their role in chronic infection is, however very complex and not entirely elucidated.[11] Reports regarding this subject are conflicting and data regarding children are limited.[6,7]

What is known

- Important role of Natural Killer (NK) cells in antiviral response.
- High proportion of NK cells situated in the liver.
- Alteration in the number, phenotype and function in hepatitis C (HCV)-infected adults.

What is new

- The expression of NK cell inhibitory CD158b and CD158e receptors related to the liver injury expressed as higher aminotransferase activity, more advanced liver fibrosis and the presence of steatosis in children with chronic hepatitis C (CHC).
- The phenomenon may be a result of direct interaction of NK cells with HCV or an underlying cause of serious liver injury.

AIM

The aim of this study was to analyze possible relation between the expression of NK cell receptors and varied intensity of liver lesions in the course of CHC in children.

MATERIALS AND METHODS

Study included 105 children with CHC - 54 boys and 51 girls, mean age 13.62 ± 3.48 years with a diagnosis of CHC based on the presence of HCV-RNA in blood for a period of time longer than 6 months. Children infected with HBV, CMV, EBV and HIV were excluded from the study as well as children with other chronic liver diseases. Duration of infection was counted from the moment of diagnosis. Children with the history of malignancy were included in the study at least 5 years after completion of oncological treatment. In 53 leukemias or lymphomas were diagnosed (chemotherapy), 17 children were treated because of solid tumors (surgical treatment). Children with CHC who underwent antiviral treatment with recombinant interferon (IFN) and ribavirin were included in the study 2 years after cessation of the therapy. All of them were non-responders. Informed consent was obtained from parents or legal guardians. Blood samples for biochemical, virological and cytometric testing was taken prior to the liver biopsy. Biochemical tests were performed on standard laboratory analyzer. HCV-RNA was detected with RT-PCR test (Amplicor HCCTM test - sensitivity level 50 IU/mL). Genotype was evaluated with Versant HCV genotype test.

Biopsy specimens were taken in 105 children in local anaesthesia with Menghini needle (Braun). Histological evaluation was preformed according to METAVIR scoring system.[12] Pathologist was blinded from history and clinical data of the patient.

Cytometric testing was performed at the time of liver biopsy NK cells were identified in patient PBMC as CD3-/CD56+, using monoclonal antibodies: anti-CD3 peridinin-chlorophyll-protein complex (PerCP, Becton Dickinson (BD) Biosciences, USA) and anti-CD56-allophycocyanin (APC Mouse anti-Human CD56, BD Biosciences, USA). The expression of NK cells surface antigens was evaluated using patient PBMC incubated with the following antibodies to inhibitory and activating receptors: anti CD158b (KIR2DL2/DL3)-phycoerythrin (PE) (human; clone DX27), anti-CD158e (KIR3DL1)-PE (human; clone DX9) and anti-CD314 (NKG2D)-PE (human; clone BAT221) (Miltenyi Biotec Inc.) Mouse IgG1-PE, Mouse IgG2a-PE, Mouse IgG2a-APC (Miltenyi Biotec Inc.) were used as control kits. Background levels of staining was determined by isotype-matched controls. FACS lysing solution was used to lyse red cells and the lymphocytes were washed three times before further analysis on a FACS Canto flow cytometer (Becton Dickinson, USA). NK cell receptors were identified by flow cytometry and the results were presented as proportion of cells and mean fluorescence rate (MFI).

The study received the approval of the Bioethical Committee of the University of Medical Sciences in Poznan, Poland (no. 234/08 from September 4, 2008).

For the purpose of analysis children were divided into different subgroups according to ALT activity (≥ 2 UNL or < 2UNL), presence of significant fibrosis (assessed ≥ 2 or < 2), presence of advanced fibrosis (assessed ≥ 3 or < 3), presence of steatosis. Control group consisted of 23 healthy children - 12 boys and 11 girls, age 13.48 ± 5.14 years ($p = 0.98$). In whom only blood for hematological parameters (WBC, HGB, PLT) cytometry and ALT and AST activity was taken.

Continuous variables were presented as mean, standard deviation, median and range. Normality was assessed using

the Shapiro-Wilk test. Consequently, Mann-Whitney test or Student's *t* test was used where appropriate. Categorical variables were expressed as frequency and percentage. They were compared by the chi-square or Fisher exact test where appropriate. Comparison of multiple groups was performed using Kruskal-Wallis test. Factors associated with liver injury were sought by univariate analysis and multivariate analysis. Features found significant in the comparatory tests were included univariate analysis. Multivariate analysis was performed by a model including factors found significant in univariate analysis. Values with $p < 0.05$ or with confidence interval (CI) not including 1.0 were defined as statistically significant.

RESULTS

Baseline group characteristic has been presented in table 1. Ninety-nine children had risk factors of pareneteral infection while six children of mother-to-child transmission. Seventy children had a history of childhood malignancy - 53 with leukemias or lymphomas (chemotherapy), 17 children with solid tumors (surgical treatment with prior chemotherapy). Moreover, fifty children underwent former antiviral treatment with null response. In 58 children with CHC (55.2%) significant liver fibrosis was observed assessed at least F2 in METAVIR scoring system. In 18 children (17.2%) advanced liver fibrosis was found. Only 5 children had no fibrotic lesions in the whole study group. Inflammatory activity was assessed A1 in 38 children (36.2%) and A2 in 50 children (47.6%). Seventeen patients had high inflammatory activity assessed A3 (16.2%). Fifty two patients had fatty liver. The results of histological assessment and were presented in figure 1. Figure 2 shows an example of advanced liver fibrosis in Syrius red staining.

Number of children with malignancy in the history did not differ significantly in the groups of children with var-

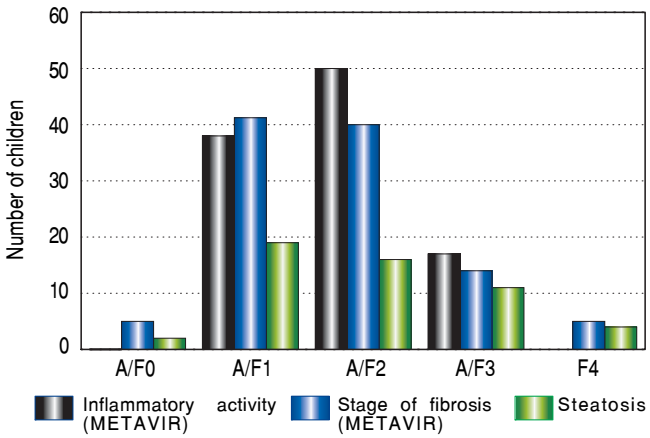

Figure 1. *Histopathology assessment - inflammatory activity, liver fibrosis and steatosis in children with CHC, n = 105.*

Table 1. Clinical and laboratory group characteristic.

Parameter	Number	Percent	$\bar{X}$ ± SD	M	Range
Age (years)	-	-	13.62 ± 3.48	15.00	2-17
Gender: M/F	54/51	50/49	-		
Age at diagnosis (years)	-	-	6.04 ± 4.23	5.00	0-16
Duration of infection (years)	-	-	7.58 ± 4.30	7.00	1-16
Probable route of infection: parenteral/vertical	99/6	94/6	-	-	-
History of malignancy Y/N	70/35	60/34			
Former antiviral treatment Y/N	50/55	47/53	-	-	-
Weight (kg)	-	-	50.62 ± 15.38	53.00	12.00-88.00
Body mass index - BMI (kg/m^2)	-	-	19.68 ± 3.39	19.45	9.92-29.41
HCV genotype 1b/1a/3a	19/80/6	19/75/6	-	-	-
HCV-RNA (IU/mL)	-	-	$1.31x106 \pm 5.75x10^6$	$2.89x10^5$	$1x\ 10^2$-$5.63x10^7$
ALT (IU/l)	-	-	64.42 ± 74.57	39	6-486
AST (IU/l)	-	-	52.95 ± 43.86	41	20-261
GGTP (IU/l)	-	-	35.25 ± 51.86	22	8-311
WBC (G/l)			6.45 ± 2.10	6.03	6.50-15.90
Inflammatory activity (METAVIR)	-	-	1.85 ± 0.85	2.00	1-3
Fibrosis (METAVIR)	-	-	1.71 ± 0.94	2.00	0-4
Liver steatosis Y/N	52/53	49/51	-	-	-

$\bar{X}$: mean. SD: standard deviation. M: median. BMI: body mass index. Y: yes. N: no. G: 10^9. IU: international unit. kg: kilogram.

ied ALT activity, liver fibrosis and liver steatosis. The results were presented in figure 3.

The strategy used to identify the expression of NK cell receptors was presented in figure 4. The number and proportion of NK cells was comparable in the group of treatment naïve and treatment experienced children. The density of CD158e expression was significantly higher in treatment naïve patients (16,990 ± 7,305 *vs*. 10799 ± 9,358 (MFI); p = 0.036). Number of NK cells was comparable in the subgroups of children with varied ALT activity (313 ± 212 *vs*. 466 ± 344 cells/μL; p = 0.123), presence of significant (342 ± 259 *vs*. 361 ± 246 cells/μL p = 0.822) and advanced fibrosis (334 ± 225 *vs*. 249 ± 210 cells/μL; p = 0.442) and liver steatosis (370 ± 309 *vs*. 315 ± 146 cells/μL, p = 0.50) (Tables 2 and 3). No statistically significant correlation were noted between the groups.

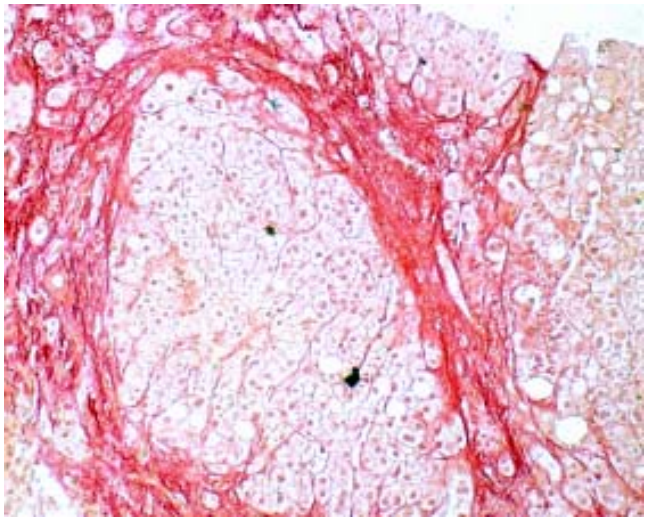

Figure 2. *Liver specimen showing fibrotic lesions surrounding liver regenerating nodule. Syrius red staining. Magnification 40x.*

Higher proportion of cells expressing CD158e inhibitory receptors was observed in the group of children with ALT > 2UNL compared to patients with low ALT activity (21.11 ± 14.60 *vs*. 12.22 ± 8.99%; p = 0.037) (Table 2). Moreover, higher proportion of cells expressing inhibitory CD158b receptor was observed in children with significant fibrosis (F ≥ 2) compared to minimal fibrosis (F < 2) - (34.14 ± 12.44 *vs*. 27.48 ± 8.71%; p = 0.049) (Table 3.). Proportion of cells with the expression of NKG2D was relatively high in all compared subgroups of children and no statistically significant differences were observed in relation to significant fibrosis.

Children with advanced fibrosis (F ≥ 3) developed higher density of CD 158b expression presented as MFI values compared to those with fibrosis assessed F < 3 (5344 ± 3407 *vs*. 2980 ± 1190; p = 0.049). Furthermore, children with coexisting steatosis had higher expression of CD158e (16908 ± 8705 *vs*. 10877 ± 8245; p = 0.035) and lower expression of NKG2D (4157.50 ± 593.34 *vs*. 5176.75± 972.41; p = 0.003).

Proportion of NK cells with the expression of CD158b was found a predictor of significant fibrosis in univariate analysis - (OR 1.065; 95%CI (1.07-1.15); p = 0.046). The density of CD158b expression was, however not found to be a predictor of advanced fibrosis in univariate analysis (OR 0.99; 95%CI (0.99-1.01); p = 0.28).

DISCUSSION

No child with CHC in this study had normal liver histology. Although liver fibrosis is a slow process, the rate of progression is difficult to predict and may be accelerated

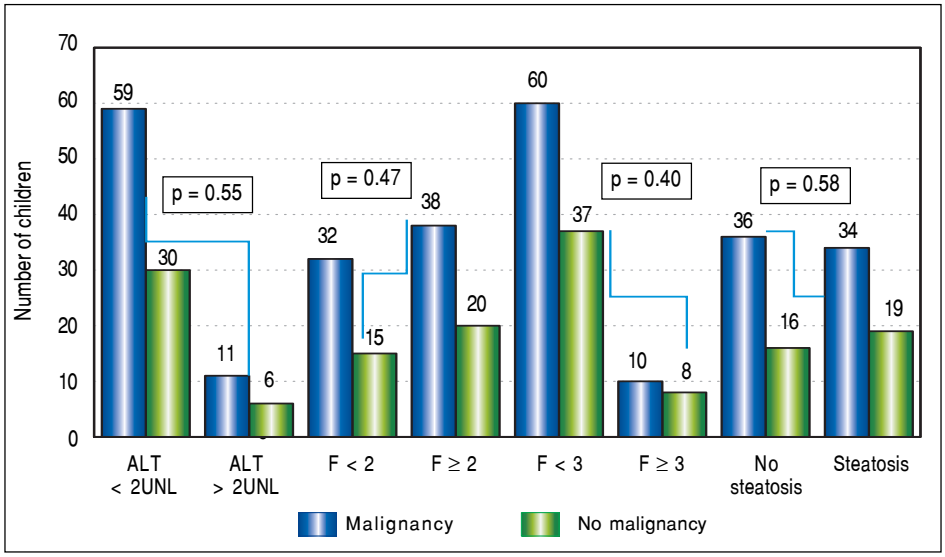

Figure 3. *Number of children with childhood malignancy in the groups with varied ALT activity, liver fibrosis and liver steatosis.*

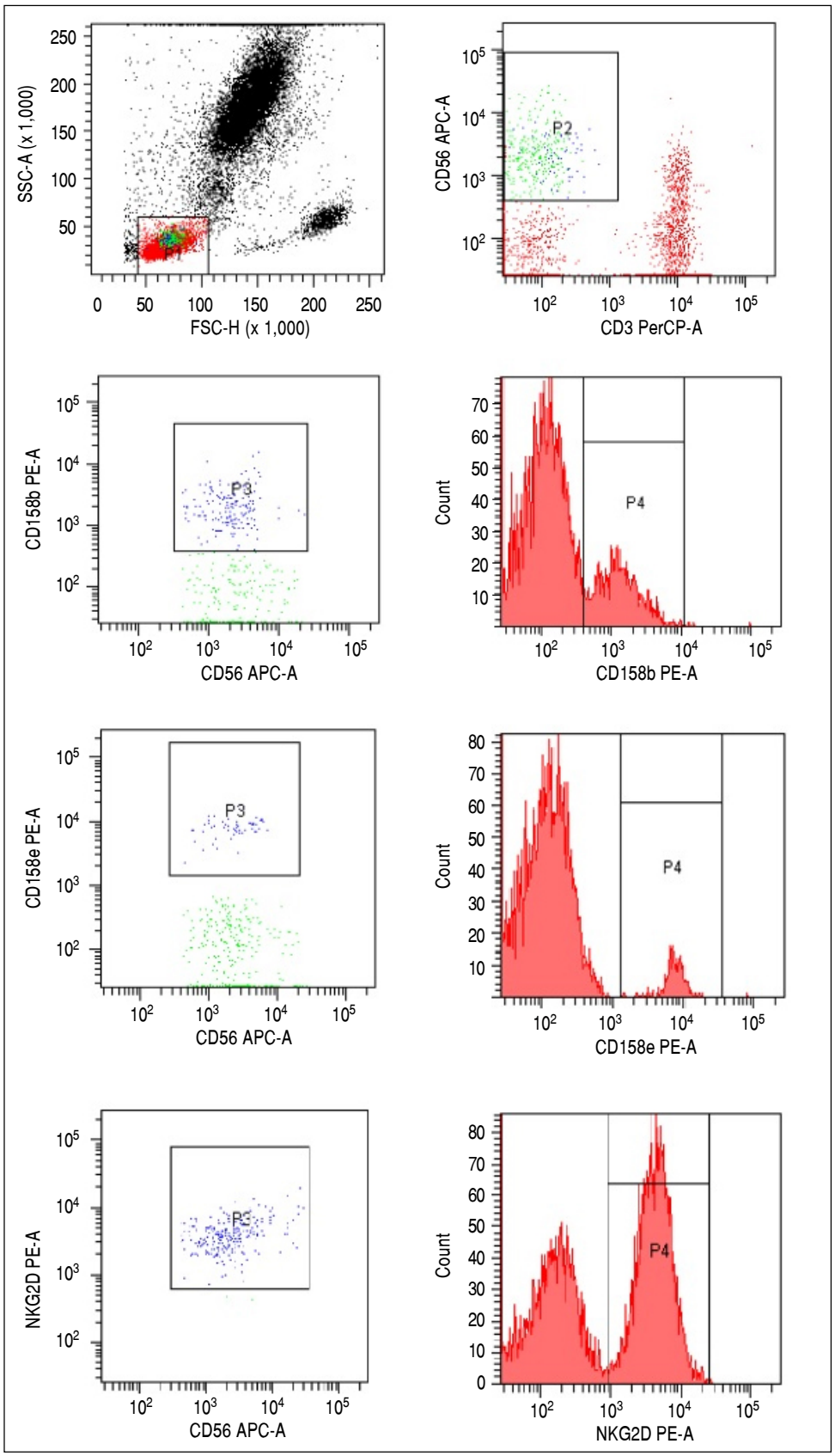

Figure 4. *Cytometric assessment of the proportion of NK cells (**A**-**B**) and the approach to evaluate the expression of selected receptors using anti-CD158b (**C**) anti CD158e (**E**) and antiNKG2D (**G**) antibodies with accompanying histograms (**D**,**F**,**H**).*

Table 2. NK cell number and expression of selected NK cell receptors in CHC group, control group, treatment naïve and experienced children and in the group with elevated and normal liver enzymes.

Feature	CHC group N = 105	Control group N = 23	p	Former antiviral treatment			ALT activity		
				Treatment naïve N = 50	Treatment experienced N = 55	p	ALT< 2 xUNL N = 88	ALT ≥ 2xUNL N = 17	p
NK (cells/μL)	345 ± 248	340 ± 164	0.93	398 ± 297	278 ± 162	0.12	313 ± 212	466 ± 344	0.12
NK cel (%)	13.79 ± 5.29	13.66 ± 5.62	0.96	13.59 ± 5.97	14.61 ± 7.27	0.65	13.83 ± 5.72	14.57 ± 9.98	0.78
CD158b (%)	30.84 ± 11.02	29.87 ± 11.18	0.74	32.74 ± 11.19	28.88 ± 11.94	0.29	29.77 ± 10.95	34.88 ± 11.03	0.25
CD158b (MFI)	3,390 ± 1914	2,962 ± 1,542	0.37	3,834 ± 1,802	3,039 ± 2012	0.22	3,277 ± 1,581	3,815 ± 2,958	0.49
CD158e (%)	14.09 ± 10.82	15.16 ± 13.32	0.73	17.28 ± 11.19	11.33 ± 10.20	0.10	12.22 ± 8.99	21.11 ± 14.60	**0.04***
CD158e (MFI)	13,575 ± 8,874	9,882 ± 8,907	0.12	16,990 ± 7,305	10,799 ± 9,358	**0.036***	12,790 ± 8,365	16,519 ± 10,667	0.30
NKG2D (%)	96.15 ± 4.44	96.70 ± 1.81	0.60	96.73 ± 3.44	95.75 ± 5.16	0.59	95.86 ± 4.87	97.06 ± 2.73	0.54
NKG2D (MFI)	4,628 ± 931	4828 ± 1402	0.56	4,503 ± 726	4,660 ± 1075	0.69	4,775 ± 930	4228 ± 874	0.19

Results presented as $\overline{X} \pm$ SD; values with p < 0.05 are considered statistically significant. CHC: chronic hepatitis C. ALT: alanine aminotransferase. UNL: upper normal limit. values with p < 0.05 are considered statistically significant.

Table 3. NK cell number and expression of selected NK cell receptors in children with varied liver fibrosis and liver steatosis.

Feature	Fibrosis score			Fibrosis score			Liver steatosis		
	F < 2 N = 47	F ≥ 2 N = 58	p	F < 3 N = 97	F ≥ 3 N = 18	p	Steatosis Absent N = 52	Steatosis Present N = 53	p
NK (cells/μL)	342 ± 259	361 ±246	0.82	334 ± 225	249 ± 210.93	0.44	370 ± 309	315 ± 146	0.50
NK cel (%)	13.74 ± 5.34	13.82 ±7.84	0.97	14.15 ± 5.13	15.24 ± 13.05	0.74	13.66 ± 5.81	14.39 ± 7.79	0.74
CD158b (%)	27.48 ± 8.71	34.14 ±12.44	**0.049***	30.21 ± 11.37	35.88 ± 13.64	0.42	30.08 ± 13.07	31.79 ± 8.10	0.64
CD158b MFI	3,154.94 ± 1,323.62	3,615.00 ±2,401.73	0.48	2,979.67 ± 1,190.64	5,344.20 ± 3,407.49	**0.049***	3079 ± 1,377	3,774.00 ± 2,412.20	0.27
CD158e (%)	13.24 ± 8.91	14.28 ±12.55	0.76	13.44 ± 9.63	15.50 ± 15.94	0.98	13.85 ± 10.24	14.38 ± 10.24	0.83
CD158e MFI	13,156.17 ± 8,154.41	13,986.89 ±9,935.34	0.78	13,631.48 ± 8,497.39	13,631.60 ± 12,692.32	0.99	10,877 ± 8245	16,907.6 ± 8,704.55	**0.04***
NKG2D (%)	94.94 ± 6.12	96.88 ±2.65	0.27	95.99 ± 5.01	95.83 ± 3.40	0.86	95.30 ± 6.06	96.94 ± 1.97	0.329
NKG2D	4,449.80 ± 984.13	4,642.80 ±854.65	0.60	4,640.82 ± 920.12	3,580.00 ± 920.92	0.12	5,177 ± 972	4157 ± 593	**0.003***

Results presented as $\overline{X} \pm$ SD; values with p < 0.05 are considered statistically significant.

by coexisting chronic diseases and obesity. Therefore, distinguishing the group of patients with accelerated liver disease out of the whole group with moderately or slowly progressing CHC seems to be very important.[2] Various factors were evaluated in children with CHC and compared to the factors established in adults. Children have relatively lower number of coexisting diseases related to sedentary lifestyle. Nevertheless, it has to be stressed, that some patients are cancer survivors which may also influence the liver histology.[14] In current study, children finished their oncological treatment at least five years prior to the inclusion to the trial, which should reduce the influence of chemotherapy on liver histology.

The study group consisted of Polish children, who were mostly nosocomialy infected with HCV but were not coinfected with other viruses. They were inhomogeneous as far as clinical, virological and histological findings were concerned. However, established alterations enabled detailed comparable analysis of various subgroups of children. Since significant differences were detected, relation between factors related to liver injury and the expression of selected receptors could be found. Significant proportion of the study group were childhood malignancy survivors, which could influence the outcome of the study. However in order to decrease the influence of underwent oncological treatment (mostly chemotherapy), children were included in the study five years after completion of the therapy. Nevertheless, it has to be stressed that in compared groups with varied liver injury, children with malignancy in the history were present with no statistically significant difference.

The innate immune response play an important role in liver infection since this organ contains larger number of NK cells than circulating peripheral blood pool. NK cells respond to antigens that are brought to the liver by blood from gastrointestinal tract. However, the intrahepatic pool needs to be evaluated directly after the biopsy. Therefore, in this study peripheral NK cells were evaluated and compared with clinical and histological findings. In the current study no significant differences were found in the number of NK cells in the subgroups of children with varied ALT activity, intensity of liver fibrosis and the presence of steatosis. Detected differences concerned however, varied expression of NK cell receptors in these groups of patients. Indolfi et al. report decreased number of CD56+CD3-NK cell in chronically HCV infected children.[15] Current study consisted of treatment naive and experienced patients in whom lower expression of CD158e was detected. Pegylated IFN was found to activate NK cells inducing their cytotoxic function, which however correlated to the treatment response.[16,17] Lower expression of the CD158e inhibitory receptor was found in treatment experienced group in spite of the lack of response to IFN used in the past. NK cells are regulated by numerous ligands through their inhibitory and activating receptors. Moreover, in the current study NK cells displayed inhibitory potential in relation to liver injury - higher proportion of cells with the expression of CD158b and CD158e receptors. The differences were statistically significant comparing groups of children with significant and mild fibrosis. Furthermore, the expression of CD158b receptor was found a predictor of significant fibrosis in univariate analysis. Although data concerning intra-hepatic NK cells are limited, Fugier, *et al*. report, however, similar findings - significantly impaired degranulation activity of intrahepatic pool of these cells in adult CHC patients with highest inflammation and fibrosis scores.[18] Regarding inhibitory influence of NK cells on the liver fibrosis, it seems obvious that in the circumstances of the inhibitory phenotype of NK cells, more advanced fibrosis of the liver was observed.[19,20]

Alterations in NK cell phenotype may be therefore a consequence of HCV infection, as well as a predisposing factor to develop CHC. Data confirming both explanations are available. HCV directly influences the grade of NK cell activation by increased expression of MHC class I proteins on the surface of the infected cells. MHC class I receptors are the ligands of NK cell inhibitory receptors, which results in the suppression of these cells. Furthermore, HCV stabilizes ligands to inhibitory receptors of NK cells, which leads to even greater inhibition and therefore, lower potential to slow down the progression of liver fibrosis.[12,14] It has been proven that HCV directly inhibits immune cells by a contact with HCV-infected hepatocytes.[20] On the other hand, altered NK cell phenotypes could cause susceptibility to viral infections, when the advantage of inhibitory receptors is observed. Studies showed that various sets of KIR receptors predispose to the development of chronic HCV infection.[21]

In the subgroup of children with liver steatosis significantly lower expression of activating receptor NKG2D was present. In other words, lack of steatosis was associated with higher expression of the receptor with activating potential. Liver steatosis may result in the decrease of NK cell activity through the higher secretion of proinflammatory cytokines. The studies conducted on animal models revealed inversely proportional relation between the liver steatosis and the number of NK cells.[22]

Lower expression of NKG2D may be explained by the activity of HCV itself, which suppresses MHC class I-related chain type A (MICA) - a ligand of this receptor.[23] Furthermore, activation of the immune system in the conditions of liver steatosis results in the higher production of TNF-alpha, IL-12 and IFN-gamma in the liver. These cytokines cause activation or stimulation of NK cell apoptosis. Moreover, the activation of NK cells results in the

inhibition of liver fibrosis, while the presence of steatosis influence on the inhibition of their activity and progression of inflammatory lesions. Previous studies confirmed that children with steatosis in the course of CHC have significantly higher inflammatory activity in histological evaluation.[24]

Number of studies suggest that although number of NK cells may be unchanged, differences between NK cell phenotype in patients with CHC and healthy controls may be noticed.[8,9,25] Few consistent finding exist in terms of change in the expression of specific receptors. Depending on the study, natural cytotoxicity receptors are being reported as either up, down or not altered,[25,10] which is a result of the heterogeneity of patients and viral factors. Although in the current study alterations between CHC patients and healthy controls were not observed, our results showed that the NK cell phenotype and possibly NK cell function may be altered in HCV-infected children in relation to various stages of liver disease, either being a cause, or a result of the phenomenon. It was suggested that, if inflammatory status remains at lower levels, NK cells display more active functions. However, if the inflammatory status of the liver increases, these functions may be significantly suppressed. It has been proposed that, either a part of NK cells become non-reactive due to the prevention of their activation in the state of inflammation, or NK cells adapt themselves to the circumstances of prolonged exposition to HCV, which leads to a decline in cytokine production and cytotoxicity in order to reduce tissue damage.[25] Animal models show that NK cells reactivity depends on the time of exposure to inflammatory state.[26] Therefore in CHC a decline in NK cell activity may favor the development of liver fibrosis. This study seem to prove this suggestions.

CONCLUSIONS

Higher proportion of NK cells with the expression of inhibitory CD158b and CD158e receptors is associated with liver injury expressed as higher aminotransferase activity, more advanced liver fibrosis and the presence of steatosis.

ABBREVIATIONS

- **ALT:** alanine aminotransferase.
- **AST**: aspartate aminotransferase.
- **CD:** cluster of differentiation.
- **CHC:** chronic hepatitis C.
- **CI:** Confidence interval.
- **HCV:** hepatitis C virus.
- **HGB:** hemoglobin.
- **IFN:** interferon.
- **KIR:** killer cells immunoglobulin-like receptors.
- **MFI:** mean fluorescence intensity.
- **NK:** natural killer.
- **N:** no.
- **PLT:** platelets.
- **TGF-β:** transforming growth factor beta.
- **UNL:** upper normal limit.
- **WBC:** white blood count.
- **Y:** yes.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest regarding to the subject of the study.

FUNDING

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COMPETING INTERESTS

On behalf of all authors, the corresponding author states that there is no conflict of interest

ETHICAL APPROVAL

The study was approved by local ethical committee of the University of Medical Sciences

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