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Non-Hematopoietic Circulating Progenitor Cells and Presence of Coronary Artery Disease in Patients with Non-Alcoholic Fatty Liver Disease

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Abstract

Background: The levels and function of circulating progenitor cells (CPCs) may be affected by chronic metabolic diseases.

Aim: To investigate the levels and functions of CPCs in patients with non-alcoholic fatty liver disease (NAFLD) and coronary artery disease (CAD).

Methods: In this retrospective study, patients (n=82) undergoing elective coronary angiography for diagnosis of CAD were included. NAFLD was defined as presence of hepatic steatosis by ultrasound in the absence of other causes of liver disease and excessive alcohol use. After coronary angiography, patients were divided into NAFLD with CAD (n=24), NAFLD without CAD (n=13), only CAD (n=31) and Non-NAFLD and Non-CAD (n=14). CPCs were quantified by flow cytometry based on the expression of ($CD34^+$, $CD133^+$, $CD34^+CD133^+$) in presence or absence of the hematopoietic marker (CD45). We assessed serum levels of angiogenic growth factors (AGFs) (pg/ml) by Multiplex assay.

Results: The levels of the $CD45\text{-}CD34^+$ and $CD45\text{-}CD133^+$ were higher in NAFLD patients with CAD (median, 15% and 2%, respectively) than NAFLD patients without CAD (median, 9% and 1%, respectively, all $p \leq 0.05$). After age adjustment, only $CD45\text{-}CD34^+$ circulating progenitor cells remain associated with increased risk of CAD in patients with NAFLD [OR: 8.71 (1.21-62.51)]. In contrast, the levels of the serum vascular endothelial growth factor-C (VEGF-C) was lower in NAFLD patients with CAD (median, 69 pg/ml) than NAFLD without CAD (median, 146 pg/ml) (p=0.01).

Conclusions: Our results indicate that high levels of non-hematopoietic CPCs and low levels of AGFs may be associated with increased risk of CAD in NAFLD patients.

Keywords: CPCs; VEGF; CD34; NAFLD; CAD

Introduction

Non-alcoholic fatty liver disease (NAFLD) is regarded as the most common form of chronic liver disease in the United States and likely globally [1,2]. NAFLD prevalence is estimated to be between 10 and 38% of the general population [3,4]. The disease is strongly associated with obesity, insulin resistance/diabetes, cardiovascular disease (CVD), and hypertension. In addition, it is considered to be the hepatic manifestation of the metabolic syndrome [5,6]. The most common cause of morbidity and mortality among NAFLD patients is CVD, and this is generally mediated by early atherosclerosis. Previous studies have suggested that a common pathogenic mechanism exists between NAFLD and CVD [7-9].

Circulating progenitor cells (CPCs) are derived mainly from the bone marrow and contribute to ongoing endothelial repair. $CD34^+$ cells are a subset of CPCs that are shown to be mobilized and recruited to the ischemic myocardial tissue. These cells then differentiate into cardiomyocytes and vascular endothelial cells, thereby restoring myocardium function [10]. CPCs are also characterized by surface expression of immature hematopoietic markers (CD133) [11] and endothelial cell marker vascular endothelial growth factor receptor (VEGFR-2) [12,13]. Consequently, the circulating cell population that contributes to postnatal neovascularization is heterogeneous (hematopoietic/endothelial progenitor cells) and displays variable morphological growth characteristics [14]. In contrast to CD34, CD133 is not found to be expressed on mature endothelial cells. $CD133^+$ $VEGFR\text{-}2^+$ cells are rarer than $CD34^+$ $VEGFR\text{-}2^+$ cells in circulation during normal conditions [15]. Both $CD34^+$ $VEGFR\text{-}2^+$ and $CD133^+$ $VEGFR\text{-}2^+$ are considered putative surface markers of CPCs. Although $CD34^+CD133^+KDR^+$ could be used as a strict phenotype of CPCs, this type of cell is infrequently found in circulation.

The recruitment of CPCs from the bone marrow to homing sites of vasculogenesis is under regulation by many factors, including chemokines and growth factors [10]. While the exact mechanism of CPC mobilization and differentiation h cells (PBMCs) were isolated using CPT as not been fully understood, recent studies have revealed that CPCs may promote local angiogenesis by secreting AGFs in a paracrine manner [16,17]. Furthermore, both CPC level and migration

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capacity could be enhanced by factors such as drugs, physical exercise, and growth factors [18-20].

The levels and functions of CPCs can be negatively impacted by pathological conditions, including diabetes mellitus [21] and CAD [16,22]. Moreover, it has been shown that individuals with lower CPC levels are at a higher risk of coronary artery disease [23]. In this study, we investigated the level and function of circulating progenitor cells in patients with NAFLD and CAD. Additionally, we hypothesize that angiogenic pathways are impaired in some patients with NAFLD, leading to an increased prevalence of coronary artery disease.

Materials and Methods

Patients and samples

In this retrospective study, the cohort consisted of 82 patients who were scheduled for elective coronary angiography because of suspected CAD**.** Following informed consent, patients underwent a coronary angiography and had a bedside hepatic ultrasound for assessment of fatty liver (both within 24 hours). Coronary angiographies were read by cardiologists to score for the diagnosis and severity of CAD using a scale of 0 to 3 based on number of major vessels with luminal stenosis ≥70% (Figure 1) [24]. NAFLD was diagnosed by the presence of steatosis using hepatic ultrasonography (Figure 2) and after exclusion of other liver diseases by serologic tests and clinical data. All hepatic ultrasounds were read by one radiologist. Clinical and laboratory data was collected for each patient, and subjects without NAFLD or CAD were considered to be controls.

Medical history and clinical data that included cardiovascular risk factors (smoking, hypertension, diabetes mellitus, hyperlipidemia, peripheral artery disease, and chronic kidney disease), previous myocardial infarction and medication were obtained. In addition, demographic, morphological and biochemical data such as body mass index (BMI), waist circumference, blood pressure and laboratory tests (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Low density lipoprotein (LDL), Total bilirubin (TBIL), Total cholesterol (TC) and High density lipoprotein (HDL) were collected. HBV and HCV were excluded by serologic tests.

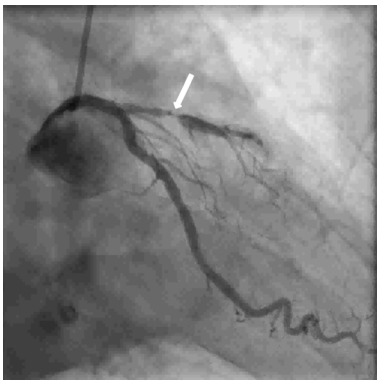

Figure 1: Coronary angiography shows right anterior oblique view of the LAD with 99% narrowing in the proximal LAD (arrow).

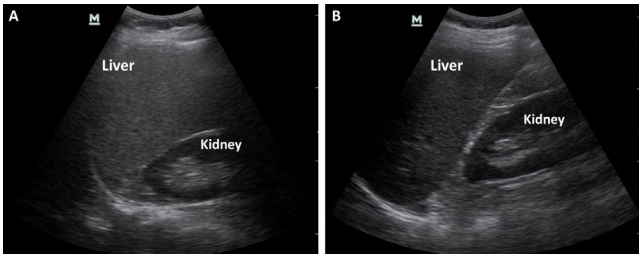

Figure 2: Hepatic ultrasonography. A) Increased echogenicity of hepatic parenchyma (NAFLD). B) Normal echogenicity of hepatic parenchyma (No NAFLD).

Blood samples were collected at the time of enrollment and sera was separated and stored at -80°C until testing. In addition peripheral blood mononuclear cells (PBMCs) were isolated using CPT tubes, frozen in cell freezing medium (Sigma-Aldrich, St. Louis, MO, USA) and kept in the liquid nitrogen until analysis.

This study was approved by the Inova Health System institutional review board.

Phenotyping of circulating progenitor cells

PBMCs were characterized for their hematopoietic/endothelial progenitor cell profiles by flow cytometry. The panels included fluorochrome conjugated monoclonal antibodies (mAbs) against the following surface antigens: CD45 (PerCp-Cy5.5), CD34 (FITC), purchased from BD Biosciences, San Jose, CA, USA; CD133(PE) from Miltenyi Biotec Inc, Auburn, CA, USA and VEGFR-2 (APC) from R&D Systems, Minneapolis, MN, USA.

Before staining with specific mAbs, frozen BPMCs were washed twice with 10% fetal calf serum in phosphate buffered saline (PBS). Cells were then resuspended in the washing buffer. Cells (1×10^6 cells) were incubated with 10µl of FITC-conjugated antihuman CD34 mAb, with 5 µl of PE-conjugated antihuman CD133 mAb, 5 µl of PE-conjugated antihuman VEGFR-2 mAb and PerCp-Cy5.5-conjugated antihuman CD45 mAb, followed by incubation at 4°C for 30 minutes. Unlabeled cells served as a control. Human mobilized peripheral blood (Department of Pathology, Inova Fairfax Hospital, Falls Church,) was used for compensation. The percentages of PBMCs positive for the above markers (CD45, CD34, CD133 and VEGFR-2) were measured by a two- side scatter-fluorescence dot plot analysis and, after appropriate gating. After exclusion of dead cells and $CD45^-$ or $CD45^+$ cells gating, CPC population was explored using dual and triple expression of CD34, CD133 and VEGFR-2. Two hundred gated cells were acquired and scored using BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

Colony-forming assay

A 0.3 ml (3×10^6 cells/mL) of isolated PBMCs was added to 3 ml of Methocult medium in duplicate in a 6 well culture plate. (MethoCult®, StemCell Technologies, Vancouver, BC, Canada). Plates were incubated at 37°C, 5% CO_2, and ≥95% humidity for 14-16 days for optimal colony forming units (CFUs) growth and morphology.

Although this conditioned medium of methylcellulose, cytokines and growth factors was optimal for granulocyte-macrophage colony-forming units (CFU-GM), macrophage colony-forming units (CFU-M), and granulocyte colony-forming units (CFU-G), it was agreed to count all of them as CFU-GM. In our study, BFU-E, CFU-GM and multi-potential progenitors (CFU-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM)) were counted using an inverted microscope. Only colonies of more than 30 cells were counted (Figure 3).

Angiogenic growth factors (AGFs) measurements

AGFs (Angiopoietin-2, Bone morphogenetic protein 9 (BMP-9), fibroblast growth factor 2 (FGF-2), Granulocyte colony-stimulating factor (G-CSF), Placental growth factor (PLGF) and vascular endothelial growth factor (VEGF-A, VEGF-B and VEGF-C) were measured using the human angiogenesis/growth factor magnetic Bead Panel 1 protocol from the Milliplex® Map Kit (Cat. No. HAGP1MAG-12K, Billerica, MA). Briefly, 96-well plates were washed with assay buffer followed by the addition of the standard, samples or controls in duplicate according to the template. Fluorescently-labeled capture antibody-coated beads were added to each well and incubated overnight at room temperature (RT) on an orbital shaker to detect AGFs. Plates were then washed 3 times using hand held plate magnet. Biotinylated detection antibodies were then added to the wells and incubated with samples for 1 hour at RT with shaking followed by the addition of streptavidin-phycoerythrin to each well and incubation for 30 minutes at RT. Finally, plates were washed and sample contents and beads were resuspended in 100 μL sheath fluid. The plates were run on the Bio-Plex® 200 System and data was collected and analyzed using the Luminex xPONENT® software (v. 4.2). Median Florescent Intensity (MFI) was applied to a 5-parameter logistic curve fitting to calculate for analyte concentration in samples.

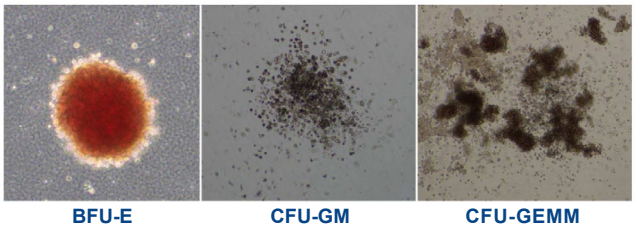

Figure 3: Photographs of different colony-forming unit. Magnifications, X10.

Statistical analysis

Mann–Whitney U-test was used to compare demographic, clinical, biochemical parameters, CPC levels and AGF levels between the different groups of patients. Pearson correlations analysis was applied to draw associations between CPC levels, AGF levels and CAD severity. Simple linear regression analyses were used to test the association between CPCs, AGFs and potential confounders. In addition, multivariate models were generated by logistic regression, after adjusting for qualified cofounders to estimate odds ratio and 95% confidence intervals for risk of CAD in NAFLD. Data are presented as median and interquartile range (IQR).

Results

Patient characteristics

The demographic, clinical and laboratory data of the study cohort are summarized in Table 1.

According to US and angiography, patients were grouped into: NAFLD with CAD (n=24), NAFLD without CAD (n=13), only CAD (n=31) and Non-NAFLD and Non-CAD (n=14). Gender (76% males), ethnicity (93% Caucasian), Smoker (44%) and use of Statin (58%) were not significantly different between the four groups. On the other hand, BMI and Triglyceride levels were significantly higher in patients with NAFLD. Twenty-six percent of the patients had clinical diabetes and 78% had hypertension.

Levels of circulating progenitor cells

Figure 4 shows the level of circulating CD45-CD34$^+$ and CD45-CD133$^+$ CPCs. The levels of the CD45$^-$CD34$^+$ and CD45$^-$CD133$^+$ were higher in NAFLD patients with CAD (median, 15% and 2%, respectively) than NAFLD patients without CAD (median, 9% and 1%, respectively, all p-values<0.05) (Figure 5A and 5B).

Characteristic	NAFLD With CAD (N=24)	NAFLD without CAD (N=13)	CAD Only (N=31)	No NAFLD No CAD (N=14)	P-value
Age (years)	65 (58-69)	55 (51-63)	67 (62-74)	53 (49-70)	<0.01
Male (%)	83	61	74	79	0.52
White (%)	96	92	87	100	0.41
BMI (kg/m2)	31 (27-35)	35 (30-38)	27 (24-29)	29 (27-31)	<0.01
Obese (BMI>30)	54	75	16	43	<0.01
Drinks (%)	56	10	27	29	0.08
Smoker (%)	33	58	46	45	0.56
Type 2 Diabetes (%)	21	54	26	7	0.04
Hyperlipidemia (%)	83	67	77	64	0.51
Hypertension (%)	83	77	87	50	0.04
Myocardial infarction (%)	0	0	16	0	0.04
Heart failure (%)	12	0	10	0	0.34
Stent (%)	4	8	29	0	0.01
Statin (%)	67	54	61	43	0.52
Glucose (mg/dL)	102 (88-118)	120 (93-307)	98 (85-109)	101 (95-113)	0.20
Alanine transaminase (U/L)	25 (21-40)	28 (22-32)	21 (16-29)	17 (15-33)	0.06
Aspartate transaminase (U/L)	23 (18-27)	20 (17-27)	23 (18-27)	20 (13-27)	0.45
Triglyceride (mg/dL)	120 (88-161)	111 (99-159)	80 (65-100)	94 (85-117)	<0.01
LDL (mg/dl)	81 (71-94)	63 (54-79)	75 (63-88)	92 (76-94)	0.13
Total Cholesterol (mg/dL)	152 (139-165)	130 (115-158)	145 (123-158)	148 (141-171)	0.28
HDL (mg/dL)	43 (36-50)	39 (36-45)	45 (34-55)	45 (30-62)	0.81
Values are presented as median and interquartile range or percentages, where appropriate. BMI-body mass index, LDL-low-density lipoprotein, HDL-high-density lipoprotein.					

Table 1: Clinical and laboratory characteristics of the study cohort.

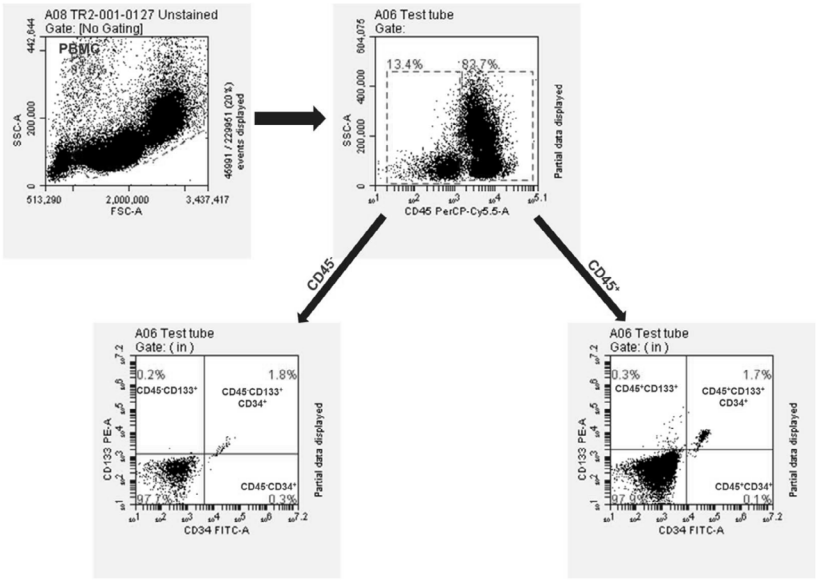

Figure 4: Representative picture of flow cytometric analysis. CPCs were quantified by flow cytometry based on the expression of (CD34, CD133,) in presence or absence of the hematopoietic marker (CD45).

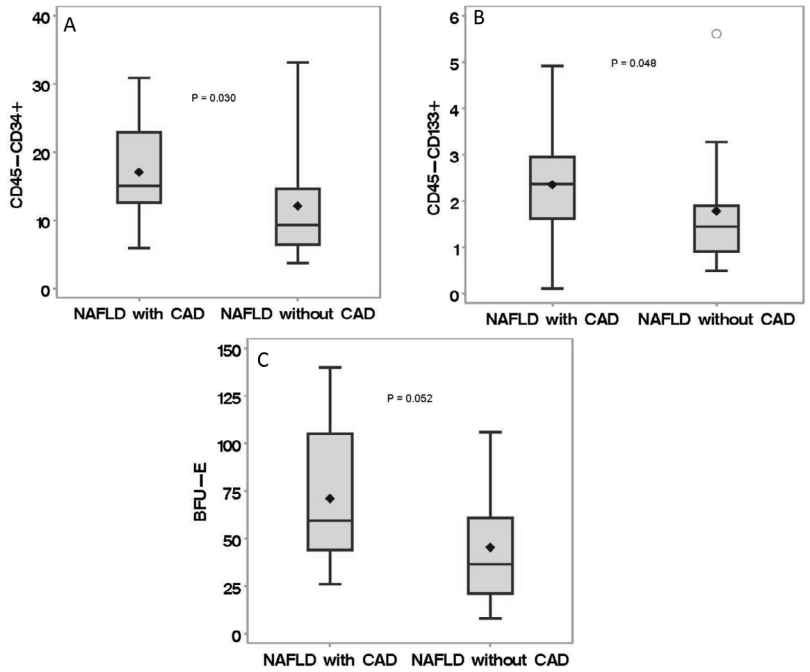

Figure 5: Levels of CPCs. A) CD45-CD34+ levels in NAFLD patients with or without CAD. B) CD45-CD133+ levels in NAFLD patients with or without CAD. C) Number of erythroid progenitors units (BFU-E) levels in NAFLD patients with or without CAD.

We also found that the levels of the CD45-CD133$^+$ were higher in NAFLD patients with CAD (median, 15%) than patients with only CAD (median, 11%, $p<0.05$). NAFLD patients in >25th quartile of CD45-CD34$^+$ and CD45-CD133$^+$ levels had the highest risk for CAD [OR: 7.00 (1.34-36.68) and 5.00 (1.07-23.46), respectively]. After age adjustment, only CD45-CD34$^+$ circulating progenitor cells remain associated with increased risk of CAD in patients with NAFLD [OR: 8.71 (1.21-62.51)] (Table 2).

Circulating progenitor cells functional capacity

The levels of the BFU-E were higher in NAFLD patients with CAD (median, 60 colonies) than NAFLD patients without CAD (median, 37 colonies, $p \leq 0.05$) (Figure 5C). NAFLD patients in >25th quartile of total CFU levels had the highest risk for CAD [OR: 7.00 (1.10-44.61)]. Furthermore patients in the lowest quartile (25th) of CFU-GM [OR: 5.92 (95% CI: 1.51-23.24)] and CFU-GEMM [OR: 5.18 (95% CI: 1.31-20.57)] were associated with increased risk for NAFLD.

Levels of angiogenic growth factors

Of the angiogenic growth factors tested, the level of serum VEGF-C was higher in NAFLD patients without CAD (median, 146 pg/ml, IQR, 58-217 pg/ml) versus NAFLD with CAD (median, 69 pg/ml, IQR, 39-86 pg/ml) versus CAD only (median, 44 pg/ml, IQR, 36-57 pg/ml) (p=0.01and p<0.01) (Figure 6A). CAD severity was inversely associated with VEGF-C (r=-0.31; p=0.01) (Figure 6B). NAFLD patients in >75th quartile of VEGF-C levels (VEGF-C >93 pg/ml) had the lowest risk of CAD [OR: 0.23 (95% CI: 0.08-0.73)] (Figure 6C).

In multivariate analysis, VEGF-C [OR: 0.26 (95% CI: 0.08-0.87)] was protective while age [OR: 1.09 (95% CI: 1.02-1.17)] was associated with increased risk for CAD. Interestingly VEGF-C was inversely associated with ALT (r=-0.3; p=0.02) and AST (r=-0.32; p=0.01). The growth factor BMP-9 was higher in NAFLD patients without CAD (median, 75 pg/ml, IQR, 49-134 pg/ml) than CAD only (median, 49 pg/ml, IQR, 21-74 pg/ml) (p=0.03).

Discussion

The association between NAFLD and CAD has been noted, but the underlying pathogenesis has not been fully investigated. Nevertheless, several hypotheses have been postulated to explain the relationship between NAFLD and CAD. Thus far, the most accepted hypothesis describes the low-grade inflammatory milieu associated with metabolic syndrome and obesity that can lead to steatohepatitis and atherosclerosis [8,25]. In addition to these inflammatory pathways, other mechanisms may also play an important role.

Variables	Univariate-adjusted OR (95% CI)	Age-adjusted OR (95% CI)
CD45-CD34+	7.00 (1.34-36.68)	8.71 (1.21-62.51)
CD45-CD133+	5.00 (1.07-23.46)	3.13 (0.56-17.45)
CFU	7.00 (1.10-44.61)	4.09 (0.55-30.28)
Values are presented as odds ratio (95% confidence interval)		

Table 2: Association between levels of CPCs and risk for CAD in NAFLD patients.

In this study, we investigated the levels and functions of CPCs in patients with NAFLD and CAD. Our data showed that the levels of the non-hematopoietic CPCs (CD45-CD34$^+$ and CD45-CD133$^+$) are higher in NAFLD patients with CAD as compared to those NAFLD patients who do not have CAD. Furthermore, these high levels of CD45-CD34$^+$ circulating progenitor cells are associated with increased risk of CAD in patients with NAFLD. These findings are in contrast to another study that showed a decrease in CPCs with cumulative cardiovascular risk [23]. Eizawa and colleagues [26] showed that diabetes is an independent predictor of low levels of CD34$^+$ cells in patients with CAD compared with age matched controls. However, in those studies, the investigators did not distinguish between CD45$^+$ and CD45- (CD34) cells (a pan-hematopoietic marker). Early-stage CPCs from bone marrow were characterized as CD45- mononuclear cells and by the expression of surface CD34, CD133, and the endothelial-specific antigen VEGFR-2 [27,28]. In this study, the VEGFR-2 surface marker was not detectable, and this could be the result of using frozen PBMCs.

These findings may support the postulation that increased CPC levels are the result of an increased hypoxia secondary to the severely stenosed coronary vessels. As such, it could be proposed that in NAFLD patients with CAD, there may be an increase in the production of CPCs. This would result in improved stimulation of angiogenesis and myocardium regeneration instead of increased risk. It also appears that increased circulating progenitor cells levels could be due to the impaired homing and differentiation capacity.

It has been reported that during ischemia VEGF promotes the development of coronary collateral vessels, thus providing adequate blood supply, preventing the death of cardiomyocytes, and aiding cardiac remodeling [29]. VEGF-C (a member VEGF family) was found to be highly expressed around the outflow tract and is important for normally patterned stems because it stimulates vessel growth near the stem sites on the aorta [30]. Furthermore, VEGF-C, when administered to animals with ischemia, promotes angiogenesis and augments blood flow to the ischemic tissues [31]. Interestingly, we also found that the levels of serum VEGF-C was lower in NAFLD patients with CAD than

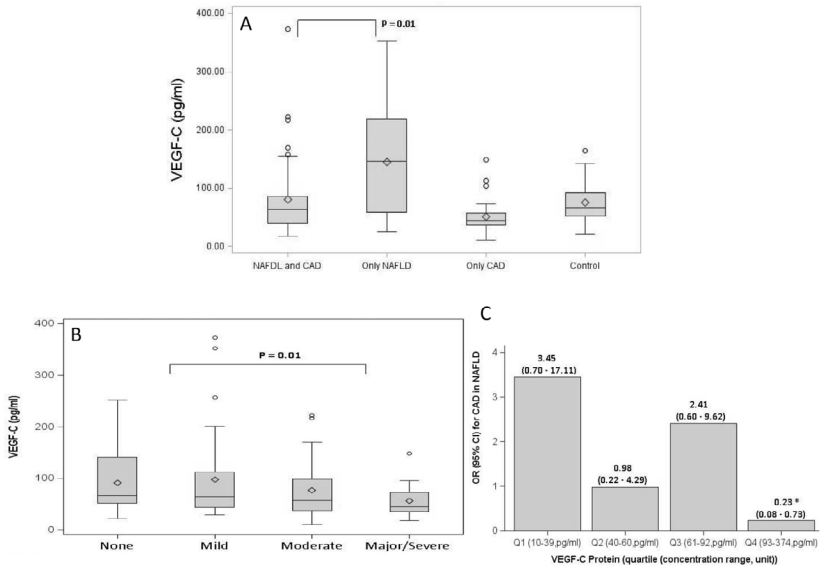

Figure 6: A) VEGF-C levels in the study groups. B) The association between VEGF-C levels and the severity of CAD in NAFLD patients. C) Association between levels of VEGF-C and risk for CAD in NAFLD patients (*p<0.05).

those NAFLD patients who did not have CAD. It was also inversely associated with the angiographic CAD severity suggesting a potential role in homing of CPCs. VEGF-C seems to play a protective role from CAD in patients with NAFLD.

It is widely accepted that there should be some correlation between VEGF levels and other risk factors for CAD such as blood pressure, diabetes, smoking, and LDL. However, in this study there was an inverse association between VEGF-C, ALT, and AST levels. This may emphasize the role of impaired liver function in the development of CAD in patients with NAFLD.

In conclusion, our study confirms that some mediators of tissue regeneration are commonly seen in NAFLD patients with CAD. Our results indicate that levels of non-hematopoietic circulating progenitor cells CD45-CD34$^+$ may be associated with increased risk of CAD in NAFLD patients. Increased circulating progenitor cell levels could be due to the low level of AGFs leading to impaired homing and differentiation capacity. Increased VEGF levels may be used to attenuate the adverse impact of NAFLD on CAD risk and improve clinical outcomes of these patients. Nevertheless, our findings would benefit from further investigation through a mechanistic functional experimental study.

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References

1. Musso G, Gambino R, Cassader M (2010) Non-alcoholic fatty liver disease from pathogenesis to management: an update. Obes Rev 11: 430-445.
2. Musso G, Gambino R, Cassader M (2011) Need for a three-focused approach to nonalcoholic fatty liver disease. Hepatology 53: 1773.
3. Angulo P (2002) Nonalcoholic fatty liver disease. The New England journal of medicine 346: 1221-1231.
4. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ (2001) Clinical features and natural history of nonalcoholic steatosis syndromes. Seminars in liver disease. 2: 17-26.
5. Ong JP, Younossi ZM (2005) Approach to the diagnosis and treatment of nonalcoholic fatty liver disease. Clinics in liver disease 9: 617-34.
6. Choudhury J, Sanyal AJ (2004) Insulin resistance and the pathogenesis of nonalcoholic fatty liver disease. Clinics in liver disease 8: 575-94.
7. Stepanova M, Younossi ZM (2012) Independent Association Between Nonalcoholic Fatty Liver Disease and Cardiovascular Disease in the US Population. Clinical Gastroenterology and Hepatology.
8. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, et al. (2006) Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 29: 1325-1330.
9. Targher G, Day CP, Bonora E (2010) Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 363: 1341-1350.
10. Rafii S, Meeus S, Dias S, Hattori K, Heissig B, et al. (2002) Contribution of marrow-derived progenitors to vascular and cardiac regeneration. Semin Cell Dev Biol 13: 61-67.
11. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, et al. (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 15: 5002-5012.
12. Ziegler BL, Valtieri M, Porada GA, De Maria R, Muller R, et al. (1999) KDR receptor: a key marker defining hematopoietic stem cells. Science 3: 285: 1553-1558.
13. Tammela T, He Y, Lyytikka J, Jeltsch M, Markkanen J, et al. (2007) Distinct architecture of lymphatic vessels induced by chimeric vascular endothelial growth factor-C/vascular endothelial growth factor heparin-binding domain fusion proteins. Circ Res 100: 1468-1475.
14. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, et al. (2004) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 104: 2752-2760.
15. Friedrich EB, Walenta K, Scharlau J, Nickenig G, Werner N (2006) CD34/CD133$^+$/VEGFR-2$^+$ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. Circ Res 17: e20-5.
16. Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, et al. (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol 24: 288-293.
17. Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, et al. (2005) Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J Mol Cell Cardiol 39: 733-742.
18. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, et al. (2002) Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 105: 3017-3024.
19. Kalka C, Masuda H, Takahashi T, Gordon R, Tepper O, et al. (2000) Vascular endothelial growth factor (165) gene transfer augments circulating endothelial progenitor cells in human subjects. Circ Res 86: 1198-1202.
20. Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, et al. (2003) Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 107: 1322-1328.
21. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, et al. (2002) Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 106: 2781-2786.
22. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, et al. (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 89: E1-7.
23. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348: 593-600.
24. Adams MR, Nakagomi A, Keech A, Robinson J, McCredie R, et al. (1995) Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. Circulation 92: 2127-2134.
25. Aller R, de Luis DA, Fernandez L, Calle F, Velayos B, et al. (2008) Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. Dig Dis Sci 53: 1088-1092.
26. Eizawa T, Ikeda U, Murakami Y, Matsui K, Yoshioka T, et al. (2004) Decrease in circulating endothelial progenitor cells in patients with stable coronary artery disease. Heart 90: 685-686.
27. Case J, Mead LE, Bessler WK, Prater D, White HA, et al. (2007) Human CD34$^+$AC133$^+$VEGFR-2$^+$ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. Exp Hematol 35: 1109-1118.
28. Timmermans F, Van Hauwermeiren F, De Smedt M, Raedt R, Plasschaert F, et al. (2007) Endothelial outgrowth cells are not derived from CD133$^+$ cells or CD45$^+$ hematopoietic precursors. Arterioscler Thromb Vasc Biol 27: 1572-1579.
29. Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, et al. (1998) Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. Circulation 98: 2800-2804.
30. Chen HI, Poduri A, Numi H, Kivela R, Saharinen P, et al. (2014) VEGF-C and aortic cardiomyocytes guide coronary artery stem development. J Clin Invest 124: 4899-4914.
31. Witzenbichler B, Asahara T, Murohara T, Silver M, Spyridopoulos I, et al. (1998) Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. Am J Pathol 153: 381-394.