Heliyon 9 (2023) e17692

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Role of circulating CD14++CD16^{+} monocytes and VEGF-B186 in formation of collateral circulation in patients with hyperacute AMI

He Zhang [a,*], Shi-lei Wang [b], Tao Sun [c], Jia Liu [d], Ping Li [e], Jing-ci Yang [f], Fang Gao [g]

[a] Department of Cardiology, The Third Hospital of Shijiazhuang City, Shijiazhuang, 050000, China
[b] Catheter Lab, The Third Hospital of Shijiazhuang City, Shijiazhuang, 050000, China
[c] Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University Beijing, 100011, China
[d] Department of Cardiology, Hebei Provincial People's Hospital, Shijiazhuang, 050000, China
[e] Department of Medical Affairs, The Third Hospital of Shijiazhuang City, Shijiazhuang, 050000, China
[f] Department of Hematology, The Second Hospital of Hebei Medical University, Shijiazhuang, 050000, China
[g] Department of Infectious Diseases, The Third Hospital of Shijiazhuang City, Shijiazhuang, 050000, China

ARTICLE INFO

Keywords:
Collateral circulation
Acute myocardial infarction
CD14++CD16^{+} monocytes
Vascular endothelial growth factor
Left ventricular remodeling

ABSTRACT

Introduction: Collateral formation is insufficient in some patients with acute myocardial infarction (AMI). Peripheral blood CD14++CD16^{+} monocytes (intermediate monocytes; IM) and vascular endothelial growth factors (VEGFs) are associated with formation of collateral circulation.
Methods: We enrolled 49 patients with AMI who underwent emergency percutaneous transluminal coronary intervention (PCI) (Group A) and 27 patients underwent delayed PCI 1 week after AMI (Group B). The percentage of circulating IM and levels of VEGFs in circulation were determined on day 8th. Left ventricular ejection fraction (LVEF) was measured 3 months after AMI.
Results: The peripheral levels of IM and serum VEGF levels on day 8th were significantly higher in patients with well-developed collateral circulation in Group A than those in Group B. The levels of circulating VEGFs in the collateral circulation (+) subgroup in Group B were lower than those in the collateral circulation (−) subgroup. Moreover, the serum VEGF-B186 levels positively correlated with IM.
Conclusions: Hyperacute collateral formation in patients with AMI correlated with a higher percentage of CD14++CD16^{+} monocytes and VEGF-B186 levels in the circulation, which was associated with milder left ventricular remodeling. The regulation of CD14++CD16^{+} monocytes and VEGF-B may be critical to the formation of collateral circulation and to healing AMI.

1. Introduction

Coronary artery occlusion leads to myocardial necrosis, resulting in impaired heart function, reduced quality of life, and even death. Standard treatments such as percutaneous transluminal coronary intervention (PCI) are not curative. Angiogenesis and arteriogenesis are two forms of neovascularization. The formation of new capillary vessels, angiogenesis, has been extensively researched and occurs in response to a hypoxic environment. Progression and expansion of already existing collateral smooth muscle-type vessels, arteriogenesis, or collateral formation, is believed to be a mechanism of organ preservation in the presence of vascular occlusion [1].

* Corresponding author.
E-mail address: ZHANGHESJZ@QQ.COM (H. Zhang).

https://doi.org/10.1016/j.heliyon.2023.e17692
Received 21 February 2023; Received in revised form 22 June 2023; Accepted 26 June 2023
Available online 29 June 2023

During myocardial infarction, collateral circulation develops in response to hypoxia and fluid shear forces [2] when heterogenous monocytes attach to local endothelial cells [3]. Findings regarding the relationship between collateral circulation and left ventricular remodeling after acute myocardial infarction (AMI) have also been contradictory.

Mononuclear macrophages play an important role in myocardial vascular repair [1]. They promote arteriogenesis through secretion of VEGF to proliferation of endothelial cells and coronary arterial smooth muscle cells [4,5]. In addition, VEGF can increase vascular permeability, resulting in monocyte infiltration. CD14++CD16^{+} monocytes, also known as intermediate mononuclear cells (IM) [6], are the main effectors of vascular and myocardial tissue repair during myocardial infarction [7], and reached peak levels on the 5th day of myocardial infarction [8]. They can be deemed as macrophages in the blood in some aspects [6]. VEGF family is a major regulator of vascular development. Animal experiments have shown that VEGF can mediate the development of collateral circulation through recruitment and activation of endothelial cells and monocytes [9]. Findings regarding VEGF have been inconsistent. Determination of the levels of VEGF-A (121,165) and VEGF-B 186 in case of AMI and the correlation of these levels with collateral circulation is critical. Furthermore, characterization of IM levels in patients who underwent emergency PCI and those who underwent delayed PCI could have an important significance in providing the basis for future exploration of improved approaches to promote arterial formation.

2. Material and methods

2.1. Study population

We enrolled 49 patients who underwent emergency PCI and 27 patients who underwent delayed PCI following diagnosis of AMI. AMI was diagnosed based upon the Fourth Universal Definition of Myocardial Infarction–chest pain that persisted for 30 min, elevation of the serum creatine kinase-MB fraction (CK-MB) to more than twice the upper limit of normal, and elevation of the serum troponin T level above the upper limit of normal according to the local quantitative or qualitative assays. Patients with ST elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI) were included. Patients with AMI who underwent emergency PCI within 12 h or within 12–24 h after symptom onset if there was evidence of continuing ischemia were classified as the hyperacute PCI group. Patients who underwent PCI on day 7th were classified as the delayed PCI group.

The criteria for exclusion from the study were the following: 1) patients with AMI who received thrombolysis. 2) patients who suffered with old MI. 3) patients with collateral formation due to a non-culprit lesion, as seen on the coronary angiography (CAG); 4) patients who previously underwent CABG.5) patients with left main and venous graft-related infarcts.6) patients with antegrade flow in the infarct-related artery at first contrast injection.7) patients whose collateral flow could not be graded for technical reasons. 8) patients with severe hepatic and renal insufficiency.9) patients with infectious diseases. 10) patients with active tumor. 11) patients with immune rheumatic diseases.12) patients with connective tissue disease.13) patients with allergic diseases.

Details regarding other inclusion and exclusion criteria are as previously published by P. Elsman et al. [10].

2.2. Approval of the study protocol

Written informed consent was obtained from all subjects prior to participation in the study. The study protocol complied with the Declaration of Helsinki.

2.3. Clinical parameters

Clinical data were obtained from a comprehensive review of patient medical records and in-hospital management records. Dual antiplatelet drugs, anticoagulant, statins, ACEI and beta blockers were prescribed when without contraindications. Echocardiography was performed at day 8th (baseline) and at 3 months post AMI as follow-up. LVEF was used as a measure of left ventricular function.

2.4. Blood sampling and analysis

Peripheral blood samples for cTNT detection were collected on admission, at 12, 24, 36, and 48 h after AMI onset. The samples were assayed locally and normalized to the upper limit of normal value. The maximum value was taken as the peak-cTNT. Peripheral blood samples were collected from all subjects on day 8th after symptom onset. Fasting cubital venous blood samples was collected in the morning and assayed immediately, with the exception of VEGF analysis. Serum samples for ELISA were aliquoted and stored at −70 °C to prevent from freeze-thaw effects.

2.5. VEGFs detection

On day 8th serum levels of VEGF-121, total VEGF-165, and VEGF-B186 were measured using commercially available ELISA kits (96 well format; R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's instructions.

2.6. CAG and PCI

The timing of CAG and PCI was determined as the duration between time of admission and time of symptom onset and thrombosis.

Patients who underwent emergency CAG and PCI within 12–24 h were included in the hyperacute PCI group. Patients were admitted later than 24 h after myocardial infarction or who underwent emergency CAG there's still heavy thrombi after treatment and without collateral formation underwent delayed PCI. Patients would be received PCI on the day 7th as the delayed PCI group. Collateral flow from the patent vessels to the infarct-related artery was graded using the classification method developed by Rentrop [11]. Based on the collateral flow grading upon first contrast injection in the contralateral coronary artery, patients were divided into three groups: Rentrop grade 0, grade 1, and grade 2/3. Anatomical SYNTAX score of the coronary tree was calculated. The thrombus aspiration technique was applied using thrombus extraction catheter and platelet glycoprotein IIB/IIIA receptor antagonist was administered to coronary artery when thrombi exist. Drug-eluting stents were implanted in the target lesion. All data were analyzed by an independent laboratory.

2.7. Flow cytometry

Percentages of monocyte subsets were measured from venous blood using a Cytomic FC500 cytometer (Beckman Coulter Inc., Miami, FL, USA) and analyzed using KLUZA software. Monocytes in 50 μl fresh EDTA anti-coagulated blood were labelled with a mixture of 10 μl anti-human antibodies CD45 PerCP (clone 2D1, BD Biosciences, CA, USA), 5 μl CD14 APC (clone RM052, Beckman Coulter, Miami, FL, USA) and 5 μl CD16 PC7 (clone 3G8, Beckman Coulter, FL, USA). After 15 min of incubation in the dark, 60 μl of hemolysin was added, and the solution was mixed in the dark for 10 min. Then, 600 μl of pure water was added, and the solution was mixed in the dark for 10 min followed by dilution in 3 ml normal saline. After a final washing step, cells were re-suspended in 0.5 ml PBS and subjected to immediate flow cytometric analysis. According to the expression levels of CD14 and CD16, monocytes were divided into three subgroups: classical (CM), intermediate (IM), and non-classical (NCM) monocytes (CM: CD14++CD16-, NCM: CD14 + CD16++, IM: CD14++CD16^{+}).

Table 1
Baseline data in AMI patients underwent emergency and delayed PCI group.

	Emergency PCI group			Delayed PCI group		
Baseline data	collateral (+) (n = 10)	collateral (−) (n = 39)	P value	collateral (+) (n = 11)	collateral (−) (n = 16)	P value
Age (years)	65 ± 8	67 ± 7	0.48	65 ± 8	66 ± 6	0.73
Male, n (%)	6 (60)	20 (51)	0.73	8 (73)	9 (56)	1.0
Previous history						
Hypertension, n (%)	4 (40)	16 (41)	0.48	4 (36)	9 (56)	0.24
Diabetes, n (%)	2 (20)	9 (23)	1.0	2 (18)	6 (38)	0.40
Hypercholesterolaemia, n (%)	4 (40)	27 (69)	0.14	3 (27)	5 (31)	1.0
Smoking, n (%)	3 (30)	11 (28)	1.0	4 (36)	3 (19)	0.39
Body Mass Index (BMI)	26.9 ± 3.4	28.3 ± 3.8	0.28	27.9 ± 3.5	28.8 ± 3.2	0.51
Family history of CAD, n (%)	1 (10)	2 (5)	0.50	1 (3)	6 (38)	0.18
Previous MI, n (%)	1 (10)	4 (10)	1.0	4 (11)	2 (5)	0.19
Previous revascularisation, n (%)	1 (10)	2 (5)	0.50	1 (3)	5 (12)	0.35
Angina, n (%)	8 (80)	18 (46)	*0.08*	8 (80)	9 (56)	0.45
Preinfarction angina, n (%)	6 (60)	17 (44)	0.48	5 (45)	3 (19)	0.19
Laboratory parameters on day 8						
Monocytes (10^9/L)	0.46 ± 0.09	0.41 ± 0.05	0.12	0.51 ± 0.10	0.46 ± 0.08	0.16
Platelet count 10^9/L	256 ± 49	258 ± 60	0.91	246 ± 45	233 ± 61	0.53
WBC count 10^9/L	7.06 ± 1.85	6.81 ± 1.53	0.70	8.30 ± 1.85	7.21 ± 1.53	0.12
Hemoglobin mmol/L	130.5 ± 14.2	129.8 ± 15.7	0.89	124.5 ± 14.8	129.6 ± 14.9	0.39
Total cholesterol mmol/L	4.76 ± 1.3	4.68 ± 0.95	0.86	4.80 ± 1.57	4.50 ± 0.76	0.53
HDL-cholesterol mmol/L	1.30 ± 0.35	1.34 ± 0.28	0.74	1.32 ± 0.39	1.24 ± 0.24	0.55
LDL-cholesterol mmol/L	3.18 ± 1.42	2.86 ± 0.63	0.50	3.18 ± 1.42	2.96 ± 0.63	0.64
Triglycerides mmol/L	1.79 ± 0.91	2.04 ± 1.33	0.49	1.49 ± 0.81	2.14 ± 1.52	0.16
Glucose mmol/L	7.20 ± 2.32	7.21 ± 2.10	0.99	7.21 ± 2.33	7.25 ± 2.91	0.97
HbA1c (%)	6.7 ± 1.2	6.4 ± 1.3	0.50	6.5 ± 1.1	6.3 ± 1.3	0.67
Creatinine umol/L	79 ± 24	87 ± 39	0.42	76 ± 29	86 ± 35	0.43
AST U/L	43 ± 38	47 ± 26	0.76	63 ± 58	37 ± 41	0.22
ALT U/L	40 ± 45	29 ± 35	0.49	52 ± 45	32 ± 31	0.22
Troponin T (ng/mL)	0.57 ± 0.39	0.66 ± 0.33	0.52	0.67 ± 0.36	0.63 ± 0.43	0.80
eGFR (ml/min)	68 ± 10	72 ± 8	0.26	70 ± 9	77 ± 8	*0.09*
In-hospital management						
Aspirin,n (%)	10 (100)	39 (100)	1.0	11 (100)	16 (100)	1.0
Clopidogrel, n (%)	8 (80)	31 (79)	1.0	8 (73)	11 (63)	1.0
Ticagrelor,n (%)	1 (10)	5 (13)	1.0	1 (9)	2 (13)	1.0
Low molecular weight heparin,n (%)	7 (70)	27 (69)	1.0	10 (91)	15 (94)	1.0
ACEI %,n (%)	5 (50)	21 (54)	1.0	5 (45)	8 (50)	1.0
β-blocker %,n (%)	6 (60)	23 (59)	1.0	7 (64)	10 (63)	1.0
Statin %,n (%)	9 (90)	36 (92)	1.0	10 (91)	15 (94)	1.0
Evaluation of severity of coronary artery lesion						
Syntax score	24.3 ± 9.7	23.6 ± 10.2	0.84	30.2 ± 10.3	23.8 ± 9.9	*0.09*

2.8. Statistical analysis

Continuously normally distributed data were expressed as the mean value ± the standard deviation. Non-parametric data were expressed as the median and the interquartile range. Analysis within groups for categorical data was performed using the chi-squared test. If more than 20% of $T < 5$ or at least 1 $T < 1$, Fisher's exact probability method was used. Analysis within groups for continuously normally distributed data was performed using Student's T test. Analysis among subgroups for statistically significant differences was performed using the Brown-Forsythe test when the variances were not equal. Pearson correlation test was used for regression analysis. Graphs were drawn with GraphPad software (GraphPad Prism 8, Inc, La Jolla, CA). Analyses were performed using SPSS 22.0 software. Data were considered statistically significant when $p < 0.05$.

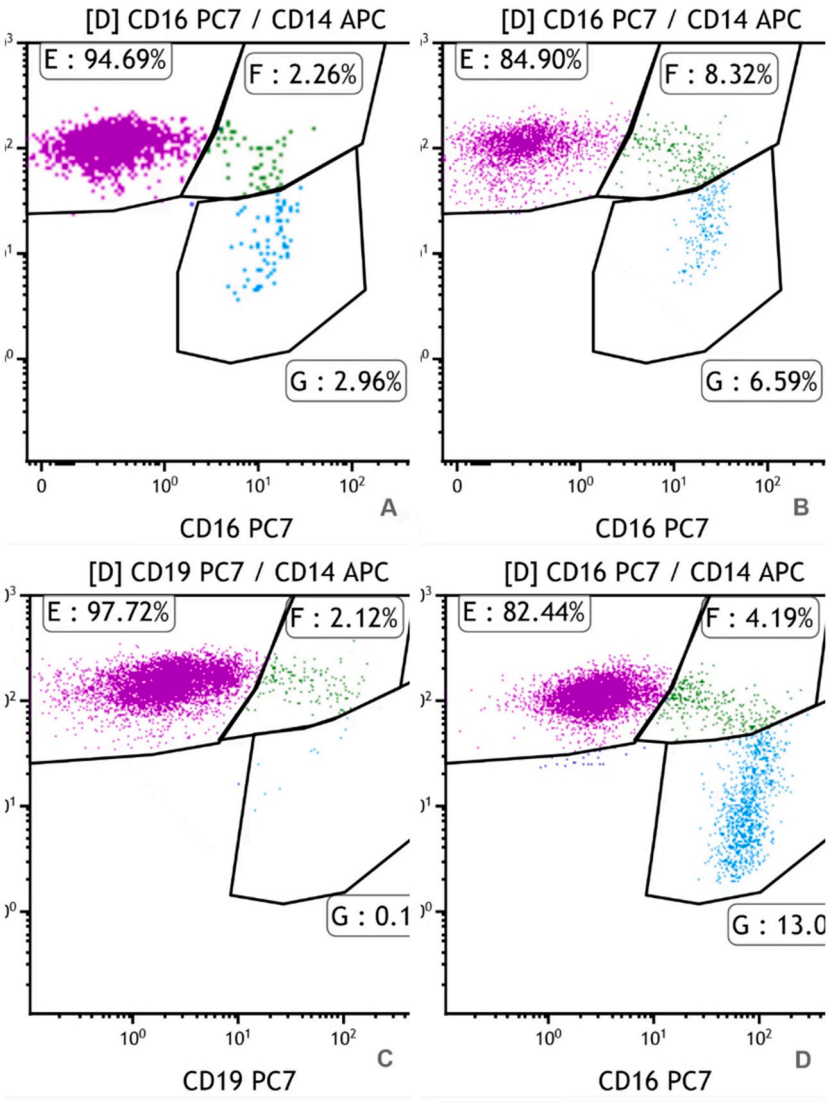


Fig. 1. Gating strategy for identification of classical (CM), intermediate (IM), and non-classical (NCM) monocytes.
After whole blood staining and red blood cell lysis, the monocyte population was defined as CD45 positive cells exhibiting a typical location in a CD45 and sideward scatter (SSC) plot. The remaining CD45 population was then distinguished according to their CD14 and CD16 surface expression identifying them as CM, IM and NCM. Representative dot plot depicting percentage of IM (shown as F) in A. collateral (+) in group A, B. collateral (−) in group A, C. collateral (+) in group B and D. collateral (−) in group B.

3. Results

3.1. Comparisons of baseline clinical characteristics

Blood samples were obtained from 49 patients with AMI who underwent emergency PCI and 27 patients with AMI who underwent delayed PCI. All of the study patients completed the trial. Baseline characteristics and blood sampling for routine laboratory parameters on day 8th and during in-hospital management are shown in Table 1. Risk factor profiles were similar among the collateral (+) and collateral (−) sub-groups within each group. Clinical parameters associated with AMI were comparable between the subgroups with AMI. There were more patients had angina for more than 3 months in the collateral (+) subgroup than in the collateral (−) subgroup in group A, but the difference was not statistically significant. Glomerular filtration rate was lower and syntax score was higher in the collateral (+) subgroup than in the collateral (−) subgroup in Group B, but the differences were not statistically significant. There were no significant differences in total number of peripheral monocytes between the collateral (+) and collateral (−) subgroups in either group, as shown in Table 1.

Difference of peripheral IM percentage between subgroups.

The gating strategy for identification of IM is shown in Fig. 1A–D. The percentage of IM was significantly lower on day 8th in the circulation of the collateral (+) subgroup than that in the collateral (−) subgroup in both group A and group B, as shown in Fig. 2A and B. The percentage of IM was significantly higher on day 8th in the circulation of the collateral (+) subgroup in group A than that in the collateral (+) subgroup in Group B (Fig. 2C).

Analysis of serum VEGFs levels in AMI patients with or without collateral circulation.

In group A, the levels of circulating VEGF-165 and VEGF-B186 in the collateral circulation (+) subgroup significantly lower than those in the collateral circulation (−) subgroup, in contrast, VEGF-121 levels were lower in the collateral (−) subgroup than those in the collateral (+) subgroup. In group B, levels of all isoforms of VEGF were significantly lower in the collateral (+) subgroup than those in the collateral (−) subgroup (Fig. 3A–B). Significantly higher serum levels of all VEGF isoforms were observed on day 8th in the circulation of the collateral (+) subgroup in group A than those in Group B (Fig. 3C).

Comparison of left ventricular remodeling 3 months post AMI in patients with or without collateral circulation.

In group A, the LVEF 3 months post-AMI in the collateral (+) subgroup was higher significantly than that in the collateral (−) subgroup and the mean LVEDD 3 months post-AMI was smaller in the collateral (+) subgroup than that in the collateral (−) subgroup, but the difference was not statistically significant. Group B showed an opposite trend. The comparison among all subgroups indicated that there were significant differences in LVEF among all subgroups, and the collateral (+) subgroup in group A had the highest LVEF level (Table 2).

Correlation between percentage of IM and VEGF-B186 levels in circulation on day 8th after AMI onset in all enrolled patients.

4. Discussion

Vascular circulatory systems were damaged in AMI patients simultaneously with myocardium. Mammals have two specialized vascular circulatory systems, the blood vasculature and the lymphatic vasculature. Blood vessels are essential for oxygen and nutrient delivery, and to expel wastes for detoxification and replenishment, while lymphatic vasculature plays essential roles in immune surveillance [12].

Previous studies have shown that collateral blood flow is an important determinant of infarct size [12]. Blood monocyte concentration is critical for enhancement of collateral artery growth [13,14] and macrophages are the key sources for VEGF during arteriogenesis [15]. Vascular endothelial growth factor facilitates infiltration and adhesion of monocytes. Our study showed that the percentage of IM and VEGF-B186 levels positively correlated, which indicated synergy between immune cells and cytokines. Our results were consistent with those in a study in which macrophage population in ischemia significantly increased arteriogenesis of adults [16]. In a multi-center controlled double-blind clinical study of REPAIR-AMI, a short-term infusion of bone-marrow-derived monocytes into the coronary artery improved the cardiac function [17]. However, the long-term effects depended on the migratory capacity of the administered bone marrow-derived cells [18]. We believe that intervention with IM is different from natural effects of

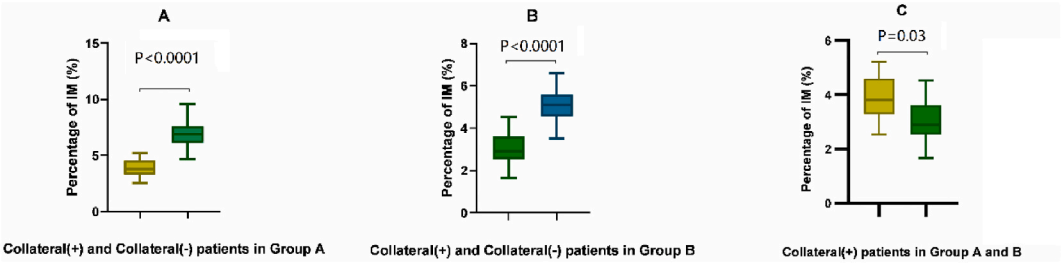
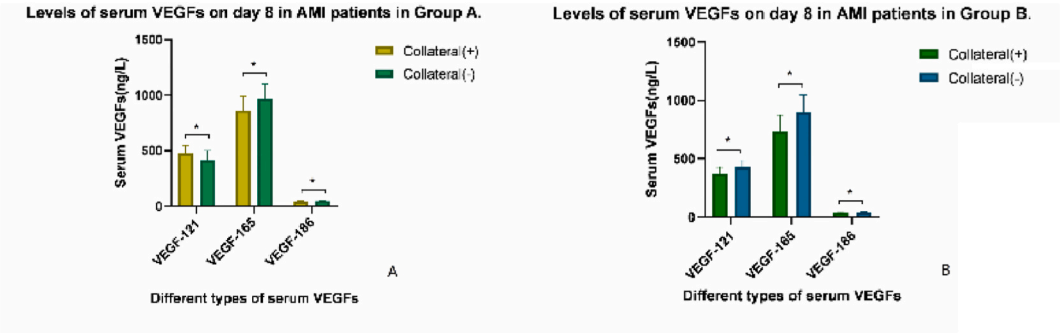


Fig. 2. Difference in peripheral percentage of IM between subgroups.
A. Between collateral (+) and collateral (−) patients in group A (n-10 vs. n = 39). B. Between collateral (+) and collateral (−) patients in group B (n-10 vs. n = 39). C. Between group A and B with collateral (+) and collateral (−) patients (n = 11 vs. n = 16).



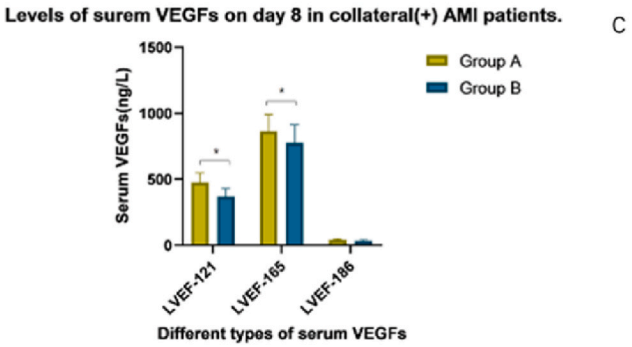


Fig. 3. A-B. Comparison of serum VEGFs levels on day 8th after AMI onset between collateral (+) and collateral (−) patients in the two groups. Group A (A. collateral (+) n = 10 vs. collateral (−) n = 39), Group B (B collateral (+) n = 11 vs. collateral (−) n = 16). *P < 0.05 collateral (+) vs. collateral (−) patients. Figure C. Comparison of serum VEGFs levels on day 8th after AMI onset between group A and group B in collateral (+) patients. (n = 10 vs. n = 11). *P < 0.05 group A+ vs. group B+.

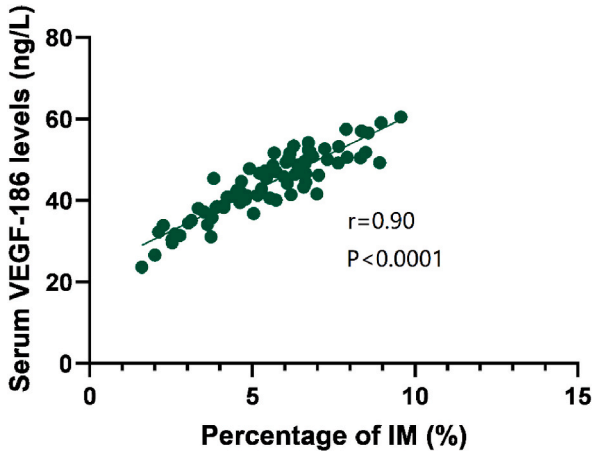


Fig. 4. Relationship between VEGF-B186 levels and percentage of IM in circulation on day 8th after AMI onset in all enrolled patients (n = 76). The percentage of IM positively correlated with VEGF-B186 levels in the circulation on day 8th after AMI onset in all enrolled patients (n = 76; Fig. 4). In contrast, the levels of VEGF-121 (r = −0.10, P = 0.85) and VEGF-165 (r = −0.45, P = 0.38) did not correlate with the percentage of IM in the circulation.

IM, perhaps because monocytes can play an adequate role in the natural enhancement due to the different immune network and microenvironment. Insufficient VEGF levels restricting its penetration may be one appearance of the mismatched microenvironments.

Animal studies showed that VEGF121 gene therapy promoted arterial genesis [19], and a small-scale clinical study also showed a

Table 2
Comparison of left ventricular remodeling variants in patients with collateral (+) and collateral (−) in group A and B.

	Group A			Group B		
Left ventricular remodeling variants	Collateral (+) (n = 10)	Collateral (−) (n = 39)	P value	Collateral (+) (n = 11)	Collateral (−) (n = 16)	P value
LVEF (%)	57.0 ± 6.9	51.8 ± 4.9	0.02	47.8 ± 8.8	52.1 ± 4.5	0.06
LVEDD (mm)	48.5 ± 3.9	50.7 ± 3.7	0.10	51.6 ± 4.0	49.2 ± 3.8	0.13

positive effect of VEGF121 gene therapy. However, some studies showed that VEGF121 induced weaker effects than VEGF165 [20]. Studies showed that VEGF-165 induced cardiac protection in large mammals with AMI by promoting arteriologenesis [21,22]. However proangiogenic therapies based on classical VEGF-A were disappointing. In addition, a study has shown that VEGF-A levels were independently associated with microvascular obstruction during STEMI and correlated with mid-term changes in LVEF [23]. Recent studies showed that VEGF-B may have potential for treatment of coronary heart disease and heart failure [24].

Levels of VEGF-B186, a diffusible isoform of VEGF-B, might be selectively active in the myocardium. Earlier studies have been contradictive. Although some studies have shown that mice lacking in VEGF-B were found to have dysfunctional coronary vasculature and impaired recovery after experimentally induced myocardial infarctions [25,26]. Others studies have shown that VEGF-B is an exception in the VEGF family, as VEGF-B does not typically lead to arteriogenesis. Studies have shown that VEGF-B may be an important survival factor for vascular endothelial cells (EC) rather than a proangiogenic factor in most organs. However, recent studies have shown that VEGF-B induced angiogenic activity in the ischemic heart in various models of pathological arteriogenesis for mice lacking VEGF-B167 or overexpressing VEGF-B167. Furthermore, VEGF-B186 induced blood vessel and collateral formation in pig and rabbit hearts. VEGF-B186 levels in the circulation were significantly lower than those of VEGF-121 or VEGF-165 in our study. VEGF-B has been shown to exert potent angiogenic, arteriogenic [27], and demonstrate antiapoptotic effects through activation of VEGFR-1 in animals subjected to myocardial infarction [10,28] Studies have also shown that VEGF receptor-1 is up-regulated to a greater degree than VEGFR-2 in response to hypoxia or oxidative stress. Treatment with adeno-associated virus (AAV9)-mediated VEGF-B186 gene therapy resulted in preservation of heart function through increased arteriogenesis [29]. The expression of transgenic VEGF-B in rat hearts has been shown to induce expansion of the coronary arterial tree, resulting in increased coronary functional reserve. Furthermore, expression of transgenic VEGF-B resulted in a shift from fatty acid utilization to glucose utilization.

A study showed that VEGF-B186 mRNA expression was significantly reduced in the boundary region and in infarcted regions of rat hearts within 42 days after myocardial infarction. Furthermore, VEGF-B has been shown to be downregulated in heart failure. The low concentration of VEGF-B186 in the circulation in our study was consistent with these findings. However, VEGF-B might play a greater role than other VEGF species in infarcted myocardium. Since IM only correlate with levels of VEGF-B186 but not with VEGF-121 or VEGF-165, this might imply that it supports VEGF-B186's role in arteriogenesis.

Some studies have shown that collateral circulation resulted in smaller infarct size, preservation of cardiac function after acute infarctions, and reduction in post-infarct ventricular dilation in patients. Our study indicated minimal left ventricular remodeling in patients with early collateral circulation formation who underwent emergency PCI, which suggested that collateral circulation in the hyperacute phase resulted in less myocardial tissue damage.

Our study showed that patients with collateral circulation had lower levels of VEGF-B186 in the circulation than those without collateral circulation on day 8th after PCI, which was inconsistent with previous findings. Lower levels of VEGF in the circulation may be due to less myocardial tissue damage. Angina pectoris prior to AMI is an important cause of collateral formation. The percentage of angina pectoris with collateral circulation who underwent emergency PCI was higher than that in patients without collateral circulation, but the difference was not significant. Left ventricular function in patients with positive collateral circulation was significantly better than that in patients without collateral circulation 3 months after AMI. Our results indicated that in patients underwent emergency PCI, less extent of damaged myocardium may account for the lower levels of VEGFs in patients with collateral circulation compared to those without collateral circulation.

Although it is controversial, formation of collateral circulation under certain conditions has been shown to be a manifestation of severe coronary lesions and is associated with poor prognosis [30]. No impact of the presence of collateral circulation is shown on delayed recanalization of the infarcted artery [31]. In the delayed PCI group, patients with collateral circulation had lower levels of VEGFs in the circulation and lower LVEF than those without collateral circulation. The presence of collateral circulation in the delayed PCI group reflected the severity of coronary lesions. The severity of coronary lesions may lead to an imbalance between the inflammatory process and the repair process, which could damage the repair process and prevent the expression of VEGF due to excessive inflammation [32]. In our study, eGFR and syntax score were higher in the collateral (+) subgroup than those in the collateral (−) subgroup in patients who underwent delayed PCI. Although not significantly different, these may also be part of the reason for the low levels of VEGF in the circulation.

5. Limitations

This study was subject to the following limitations. First, MI is also a trigger for cardiac lymphangiogenesis [33–35] in humans and mice [34,36,37]. Lymphangiogenesis modulates the inflammatory response to injury [38,39] by increasing the clearance of fluid and immune cells as well as inflammatory mediators from the injured heart to draining lymph nodes [40] to facilitate tissue remodeling and wound healing [41]. Cardiac lymphangiogenesis, as a promising and challenging field, should be a key aspect in our follow-up

studies. Second, no healthy controls were enrolled since previous studies had documented changes in VEGF expression in AMI patients. In addition, the proportion of patients with collateral circulation was low, therefore the number of enrolled patients was relatively low. Study with larger sample size is needed to further confirm it in the future. In the follow-up trial for further observation the correlation between VEGF-B186 and IM in AMI patients. Finally, this was a non-interventional study, and studies of interventions should be performed in the future.

6. Conclusion

Our study showed that hyperacute collateral formation in patients with AMI induced beneficial effects and correlated with higher levels of VEGF-B186, VEGF-165, and percentage of IM in the circulation. VEGF-B186 levels in the circulation were correlated with IM in all patients with AMI, which may indicate its role in arteriogenesis. The presence of collateral circulation in patients who underwent delayed PCI may only reflect the severity of coronary lesions. The regulation of IM and VEGF-B may be critical to the formation of collateral circulation and to the healing from AMI. Future studies should focus on promoting IM recruitment and VEGF-B186 secretion. Findings from our study should provide the basis for future exploration of improved approaches to promote arterial formation. Furthermore, future work should focus on identifying factors that promote IM recruitment and VEGF secretion in the natural pathological state because this could facilitate collateral formation in the hyperacute phase to improve the prognosis of particular patients with myocardial infarction.

Ethics statement

Informed consent was obtained from each patient and the study protocol was reviewed and approved (ethics approval number:201906) by the local human research ethics committee of the Third Hospital of Shijiazhuang. (Shijiazhuang, China).

Funding statement

This study was supported in part by the Science and Technology Development Center, Ministry of Education of China, Beijing, (Grant NO. 2021ITA05003), a project of the Industry-University-Research Innovation Fund for Chinese Universities.

Author contribution statement

He Zhang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shi-lei Wang: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Jia Liu: Performed the experiments.

Ping Li: Fang Gao: Tao Sun: Contributed reagents, materials, analysis tools or data.

Jing-ci Yang: Performed the experiments; Analyzed and interpreted the data.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

BMI: body mass index. CAD: coronary artery disease, Angina: history of angina pectoris before myocardial infarction was more than 3 months. Preinfarction angina: angina was present 48 h prior to the onset of myocardial infarction. ACEI: angiotensin-converting enzyme; ALT: alanine aminotransferase; AST: aspartate aminotransferase; cTNT: cardiac troponin T; eGFR: estimated glomerular filtration rate; SYNTAX: synergy between percutaneous coronary intervention with taxus and cardiac surgery.

Values less than $P < 0.1$ are presented in italics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thanks to Hai-xia Li for her deliberate language polishing.

References

[1] A.S. Jaipersad, et al., The role of monocytes in angiogenesis and atherosclerosis, J. Am. Coll. Cardiol. 63 (1) (2014) 1–11.
[2] F. Pipp, et al., Elevated fluid shear stress enhances postocclusive collateral artery growth and gene expression in the pig hind limb, Arterioscler. Thromb. Vasc. Biol. 24 (9) (2004) 1664–1668.
[3] M. Arras, et al., Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb, J. Clin. Invest. 101 (1) (1998) 40–50.
[4] P. Ancuta, et al., Fractalkine preferentially mediates arrest and migration of CD16+ monocytes, J. Exp. Med. 197 (12) (2003) 1701–1707.
[5] Y.X. Lv, et al., VEGF-A and VEGF-B coordinate the arteriogenesis to repair the infarcted heart with vagus nerve stimulation, Cell. Physiol. Biochem. 48 (2) (2018) 433–449.
[6] L.M. Carlin, et al., Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal, Cell 153 (2) (2013) 362–375.
[7] B. Passlick, D. Flieger, H.W. Ziegler-Heitbrock, Identification and characterization of a novel monocyte subpopulation in human peripheral blood, Blood 74 (7) (1989) 2527–2534.
[8] M. Nahrendorf, et al., The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions, J. Exp. Med. 204 (12) (2007) 3037–3047.
[9] Z. Arany, et al., HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha, Nature 451 (7181) (2008) 1008–1012.
[10] M. Rasanen, et al., VEGF-B promotes endocardium-derived coronary vessel development and cardiac regeneration, Circulation 143 (1) (2021) 65–77.
[11] K.P. Rentrop, et al., Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects, J. Am. Coll. Cardiol. 5 (3) (1985) 587–592.
[12] C.J. Bruce, et al., Determinants of infarct size after thrombolytic treatment in acute myocardial infarction, Am. J. Cardiol. 83 (12) (1999) 1600–1605.
[13] R. Zbinden, et al., Coronary collateral flow and peripheral blood monocyte concentration in patients treated with granulocyte-macrophage colony stimulating factor, Heart 90 (8) (2004) 945–946.
[14] M. Heil, et al., Blood monocyte concentration is critical for enhancement of collateral artery growth, Am. J. Physiol. Heart Circ. Physiol. 283 (6) (2002) H2411–H2419.
[15] N. van Royen, et al., Effects of local MCP-1 protein therapy on the development of the collateral circulation and atherosclerosis in Watanabe hyperlipidemic rabbits, Cardiovasc. Res. 57 (1) (2003) 178–185.
[16] Y. Takeda, et al., Macrophage skewing by Phd2 haplodeficiency prevents ischaemia by inducing arteriogenesis, Nature 479 (7371) (2011) 122–126.
[17] V. Schachinger, et al., Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial, Eur. Heart J. 27 (23) (2006) 2775–2783.
[18] B. Assmus, et al., Long-term clinical outcome after intracoronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival, Eur. Heart J. 35 (19) (2014) 1275–1283.
[19] C.A. Heilmann, et al., Transmyocardial laser revascularization combined with vascular endothelial growth factor 121 (VEGF121) gene therapy for chronic myocardial ischemia–do the effects really add up? Eur. J. Cardio. Thorac. Surg. 23 (1) (2003) 74–80.
[20] N.M. van den Akker, et al., Developmental coronary maturation is disturbed by aberrant cardiac vascular endothelial growth factor expression and Notch signalling, Cardiovasc. Res. 78 (2) (2008) 366–375.
[21] M. Ferrarini, et al., Adeno-associated virus-mediated transduction of VEGF165 improves cardiac tissue viability and functional recovery after permanent coronary occlusion in conscious dogs, Circ. Res. 98 (7) (2006) 954–961.
[22] A. Crottogini, et al., Arteriogenesis induced by intramyocardial vascular endothelial growth factor 165 gene transfer in chronically ischemic pigs, Hum. Gene Ther. 14 (14) (2003) 1307–1318.
[23] R. Garcia, et al., VEGF-A plasma levels are associated with microvascular obstruction in patients with ST-segment elevation myocardial infarction, Int. J. Cardiol. 291 (2019) 19–24.
[24] R. Mallick, S. Yla-Herttuala, Therapeutic potential of VEGF-B in coronary heart disease and heart failure: dream or vision? Cells 11 (24) (2022).
[25] D. Bellomo, et al., Mice lacking the vascular endothelial growth factor-B gene (Vegfb) have smaller hearts, dysfunctional coronary vasculature, and impaired recovery from cardiac ischemia, Circ. Res. 86 (2) (2000) E29–E35.
[26] Z.K. Otrock, J.A. Makarem, A.I. Shamseddine, Vascular endothelial growth factor family of ligands and receptors: review, Blood Cells Mol. Dis. 38 (3) (2007) 258–268.
[27] J.E. Lahteenvuo, et al., Vascular endothelial growth factor-B induces myocardium-specific angiogenesis and arteriogenesis via vascular endothelial growth factor receptor-1- and neuropilin receptor-1-dependent mechanisms, Circulation 119 (6) (2009) 845–856.
[28] L. Zentilin, et al., Cardiomyocyte VEGFR-1 activation by VEGF-B induces compensatory hypertrophy and preserves cardiac function after myocardial infarction, Faseb. J. 24 (5) (2010) 1467–1478.
[29] J. Huusko, et al., AAV9-mediated VEGF-B gene transfer improves systolic function in progressive left ventricular hypertrophy, Mol. Ther. 20 (12) (2012) 2212–2221.
[30] R.H. Helfant, P.S. Vokonas, R. Gorlin, Functional importance of the human coronary collateral circulation, N. Engl. J. Med. 284 (23) (1971) 1277–1281.
[31] P.G. Steg, et al., Impact of collateral flow to the occluded infarct-related artery on clinical outcomes in patients with recent myocardial infarction: a report from the randomized occluded artery trial, Circulation 121 (25) (2010) 2724–2730.
[32] K. Sun, Y.Y. Li, J. Jin, A double-edged sword of immuno-microenvironment in cardiac homeostasis and injury repair, Signal Transduct. Targeted Ther. 6 (1) (2021) 79.
[33] Y. Ishikawa, et al., Lymphangiogenesis in myocardial remodelling after infarction, Histopathology 51 (3) (2007) 345–353.
[34] L. Klotz, et al., Cardiac lymphatics are heterogeneous in origin and respond to injury, Nature 522 (7554) (2015) 62–67.
[35] K. Nakamura, S.G. Rockson, The role of the lymphatic circulation in the natural history and expression of cardiovascular disease, Int. J. Cardiol. 129 (3) (2008) 309–317.
[36] O. Henri, et al., Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction, Circulation 133 (15) (2016) 1484–1497. ; discussion 1497.
[37] F. Tatin, et al., Apelin modulates pathological remodeling of lymphatic endothelium after myocardial infarction, JCI Insight 2 (12) (2017).
[38] K. Klaourakis, J.M. Vieira, P.R. Riley, The evolving cardiac lymphatic vasculature in development, repair and regeneration, Nat. Rev. Cardiol. 18 (5) (2021) 368–379.
[39] E. Brakenhielm, A. Gonzalez, J. Diez, Role of cardiac lymphatics in myocardial edema and fibrosis: JACC review topic of the week, J. Am. Coll. Cardiol. 76 (6) (2020) 735–744.
[40] T. Vuorio, A. Tirronen, S. Yla-Herttuala, Cardiac lymphatics - a new avenue for therapeutics? Trends Endocrinol. Metabol. 28 (4) (2017) 285–296.
[41] T. Vuorio, A. Tirronen, S. Yla-Herttuala, Cardiac lymphatics - a new avenue for therapeutics? Trends Endocrinol. Metabol. 28 (4) (2017) 285–296.