Effects of arterial blood on the venous blood vessel wall and differences in percentages of lymphocytes and neutrophils between arterial and venous blood

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Abstract
Vascular sclerosis mostly occurs in arteries and is mainly related to anatomic structure and hemodynamics of artery. This study aimed to investigate effects of arterial blood on vein wall and explore differences of composition between arterial and venous blood.

Ultrasound was used to examine the distal venous structure of arteriovenous fistula in uremia patients. Immunohistochemistry was used to study the pathology of the distal vein. Twelve patients were divided into control group and trial group. Patients received an arteriovenous fistula within 1 month in control group. Patients had undergone this surgery ≥2 years before in the trial group. Blood samples were collected from the aortic, arterial, and venous vessels of 51 patients who had taken coronary angiography and analyzed with blood routine rest, biochemical, and immunological measures to compare the differences of blood composition between artery and vein. This study was registered with the China Clinical Trial Center website under registration number ChiCTR-OOC-16008085.

In the trial group, the vascular wall of distal veins of fistula were thickened and hardened. No significant differences of blood composition were found between the aortic and radial arterial blood. However, the differences in the percentages of lymphocytes and neutrophils between arterial and venous blood were significant (\(P_a = .0095\), \(P_b = .01\)).

Under smooth hemodynamic conditions, arterial blood caused hardening of the venous wall. Arterial and venous blood differed in the percentage of lymphocyte and neutrophils. This may contribute to the vascular sclerosis that is observed in arteries more often than veins.

Keywords: biochemistry complete set, blood components, blood routine, complete immunity, hemodynamics, lymphocyte percentage, vascular sclerosis

1. Introduction
Atherosclerosis refers to vascular wall thickening and hardening, loss of elasticity, and narrowing of lumen.\(^\text{[1,2]}\) Angiosclerosis can decrease the blood supply to organs, including heart, brain, and kidney, and subsequently cause organ ischemia and dysfunction. Atherosclerosis occurs mostly in the arteries, but rarely in the veins.\(^\text{[3]}\) This is attributed to differences in the anatomical structure of arteries and veins, as proposed by the most relevant studies.\(^\text{[4–6]}\) However, vascular sclerosis rarely occurs in the pulmonary artery, which contains venous blood only.\(^\text{[7,8]}\) This has led to a hypothesis that vascular sclerosis was associated with the particular characteristics of arterial blood. Indeed, a number of studies have suggested that the differences in physiological function and hemodynamic environment between arteries and veins contribute to the development of vascular sclerosis.\(^\text{[9,10]}\)

Recently, a number of studies showed that vein grafts used in coronary artery bypass grafting also underwent hardening, similar to original coronary arteries.\(^\text{[11,12]}\) The probability of lesion and occlusion arising in the postoperative venous bridge was reportedly ∼15% to 30% at 1 year \(^\text{[13,14]}\) and ∼50% of vein grafts failed within 10 to 15 years after surgery, due to a number of issues, including intimal hyperplasia.\(^\text{[15]}\) Intimal hyperplasia occurred mainly as a response to higher arterial pressures after the vein graft bypass surgery,\(^\text{[16]}\) and was linked to vascular wall thickening and narrowing.\(^\text{[17]}\) Hence, changes in the vascular hemodynamics in the bridge vessel can promote vascular sclerosis. In addition, it is well known that the lipid composition of the blood contributes to the pathogenesis of atherosclerosis.\(^\text{[18]}\) However, whether the differences in blood composition between arterial and venous blood are factors that influence angiogenesis is not known.

In the present study, we explored whether arterial blood components were associated with hardening of the venous wall in addition to hemodynamics, and differences in the composition of arterial and venous blood.
2. Methods

2.1. Study design

This study was approved by the Institutional Ethics Committee of the Third Xiangya Hospital of Central South University. All of the patients provided signed informed consent before their enrollment in the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was registered as Chinese Clinical Trial Registry No. ChiCTR-OOC-16008085.

2.2. Subjects

The subjects for our study are included 2 phases; in the first phase, 12 uremia patients were selected who had undergone arteriovenous fistulation to explore whether arterial blood components were associated with hardening of the venous wall in addition to hemodynamics; in the second phase, 58 patients were recruited to detect the differences in the composition of arterial and venous blood.

The first phase of the retrospective study included 12 uremia patients who had undergone arteriovenous fistulation sometime between November 27, 2015, and January 20, 2017. These patients were divided into those who had had surgery within 1 month (control group) and those who had undergone surgery ≥2 years before (trial group). Arteriovenous ultrasonography was conducted on their arteriovenous fistulas. If the patients had undergone fistulation or refistulation because of vascular occlusions, vein vessels on the surgical areas were taken for pathological examination.

The second phase of the study included patients aged 18 to 75 years in whom both arterial and venous blood were readily collectible. The participants consisted of patients with cardiovascular diseases who required coronary angiography. Patients with any of the following were excluded from the present analysis: taking statins within the recent 3 months; acute infection; malignant tumor; intractable hypertension or arrhythmia; thyroid disease; or use of systemic steroids or cyclosporine therapy. This study began on November 27, 2015, and ended September 30, 2016. Among the initial 58 patients enrolled, 4 failed to complete the blood collection process and were excluded from the study, and 3 were excluded due to the hemolysis test results. Thus, 51 patients participated in the second phase of the study. Blood samples were collected from the following locations: the radial artery’s outer periphery during coronary angiography with implantation of radial artery sheath; the aortic sinus when the angiography catheter was inserted into the aortic sinus; and a left arm vein.

Data were regularly reviewed by the independent China Clinical Trials Registry. Raw data were audited and published by the ResMan Clinical Trial Public Management Platform for Research. As the study collected blood and pathological specimens from the sampled patients, all patients involved in the study have signed the informed consent.

2.3. Research method

The PHILPS epiq7c color Doppler ultrasound system was used for vascular ultrasound examinations, and hematoxylin and eosin (H&E) staining was used for immunohistochemical examination.

During the coronary angiography, 6mL of blood were collected from each of the following: the radial artery, aortic sinus, and peripheral veins. The blood specimens were sent for laboratory tests, including routine blood, liver and kidney function, blood lipid, blood glucose, electrolyte, and complete immunity tests.

2.4. Statistical analysis

The second phase of the study analyzed the data from the blood specimens. The data from these 3 groups of specimens (aortic sinus, radial artery, and peripheral vein) were compared using analysis of variance and the Bonferroni post-hoc test for 2-group comparison. All descriptions of the test results are presented as mean±standard deviation, and a P value <.05 was considered statistically significant.

3. Results

3.1. Hardening and thickening intima of veins in patients of trial group

We selected uremia patients who had undergone arteriovenous fistula surgery within 1 month (control group) or ≥2 years previously (trial group) for vascular ultrasonographic examination. In the normal arteries of patients, blood flow was fast, and the blood flow spectrum was a serrated or wavy waveform. In veins, the blood flow was smooth and slow, and the blood flow spectrum waveform was continuous.

In the patients in the trial group, the blood flow around the fistula opening was fast, and its blood flow spectrum was also a serrated waveform. On a site far away from the fistula opening, the blood flow spectrum was similar to the continuous waveform of the venous blood flow. This suggests that hemodynamically, the blood flow had restored the venous blood flow.

Currently, in artificial fistulation, a narrow vascular lumen is considered if the peak systolic velocity at the fistula opening is ≥2.5 times that of the arterial blood inflow 2cm from the fistula, or if the diameter stenosis rate of the fistula ≥50%.[19] In the present study, the intimal thickness of the veins 5cm away from the fistula in the control group for a short time after surgery was normal (Fig. 1 A, B). However, in the trial group, the vascular ultrasound showed that the blood flow spectrum for veins ≥5cm away from the fistula opening registered as a continuous waveform, and the vascular intima was hardening as well as thickening (Fig. 1C, D).

3.2. Long-term fistulation promotes venous wall hardening and thickening

Next, we selected veins behind the fistula for immunohistochemical examination. In the control group, the venous wall contained a few layers of endothelial cells (Fig. 2 A). However, in patients of the trial group, the endothelial cells of the venous wall were thick, and mucoid degeneration of the venous wall, fiber hyperplasia, and fibroblast proliferation in the venous wall were obvious. In addition, proliferation of small vessels occurred outside the vascular wall, and the vascular intima was thickened and hardened (Fig. 2 B). This suggests that long-term fistulation promotes venous wall hardening and thickening.

3.3. Compositions of blood from the aortic sinus, radial artery, and peripheral vein

We performed a complete analysis of the composition of the blood specimens collected from the aortic sinus, radial artery, and peripheral vein (Table 1). The complete immunity tests showed no statistically significant differences among the 3 sites of
Figure 1. Typical color Doppler ultrasound images of the distal end of the arteriovenous fistula. (A and B) Control group; (C and D) Trial group. (C cf. A) The longitudinal section of the blood vessel shows the upper vein wall becoming thicker two years ago when the blood flows in the continuous spectrum. (D cf. B) The transverse section of the blood vessel shows the upper vein wall becoming thicker 2 years ago and shows the vein vessel diameter enlarging in the trial group.

Figure 2. Immunohistochemical images (H&E staining) of the venous vessels at the fistula opening. (A) The fistula opening vein vessel, control group (10 × 10). (B) Fistula opening vein vessel (10 × 40), control group. (C) Fistula opening vein vessel (10 × 10), trial group. (D) Fistula opening vein vessel, trial group (10 × 40).
collection, while the complete biochemical examinations indicated a significant difference in the glycemic index among the 3 groups ($F=4.33, P = .0152$).

Further pairwise comparisons using the Bonferroni post-hoc test revealed that the glycemic value of blood from the peripheral vein group was significantly lower than that of the aortic sinus group ($P = .0277$). The glycemic value in the peripheral vein group was also lower than that of the radial artery group, although the difference was statistically insignificant ($P = .054$). In addition, the percentage of lymphocytes from these 3 groups showed statistically significant differences ($F=4.82, P = .0095$). Further comparisons using the Bonferroni post-hoc test revealed that the percentage of lymphocytes in the blood of the peripheral vein group was significantly lower than that of the aortic sinus group ($P = .0167$) or radial artery group ($P = .0336$).

In addition, there was a significant difference in the percentages of neutral granulocytes among these 3 groups ($F=4.77, P = .01$). Similar to the results for the percentage of lymphocytes, Bonferroni post-hoc analysis revealed that the percentage of neutral granulocytes of the peripheral vein group was significantly higher than that of the aortic sinus group ($P = .0165$) or the radial artery group ($P = .0382$). Thus, we conclude that the major difference in the compositions of arterial and venous blood is the percentages of lymphocytes and neutral granulocytes.

4. Discussion

There were 3 major findings obtained from the present study. In the trial group of patients who had undergone arteriovenous fistula surgery ≥2 years previously, vascular sclerosis occurred in the wall of the vein ≥5 cm away from the fistula opening. Second, in this group, also the vein under the fistula had obviously thickened and hardened. Finally, arterial blood contained a higher percentage of lymphocytes, and a lower percentage of neutral granulocytes, than venous blood. This may potentially contribute to the development of angiosclerosis, in addition to hemodynamics.

In the present study, we first conducted ultrasonography to examine the vessel walls in uremia patients who had undergone a venous fistula operation, either ≤1 month before (control group), or ≥2 years previously (trial group). We observed that in patients of the trial group, vascular sclerosis occurred in the walls of veins
Development of vascular sclerosis, our important role for hemodynamic changes after connected. On the basis of the recycling path of lymphocytes, we thus, the lymphatic circulation and blood circulation are closely connected. Postcapillary micro veins, some lymphocytes from the arterial blood may be transmitted to the lymphatic circulation, causing a change in the percentage of lymphocytes in the veins.

There are some limitations present in this study. While the sample size was small, the difference in the percentages of lymphocytes between arterial and venous blood was significant and consistent. In addition, the study only empirically indicated that lymphocytes might have an essential role in causing atherosclerosis. Further studies with a large cohort will be needed to provide further evidence that may directly link elevated lymphocyte levels in arterial blood to atherosclerosis.

In conclusion, we have demonstrated that arterial blood has a substantially higher percentage of lymphocytes, but a lower percentage of neutrophils, compared with venous blood. This is potentially responsible for the more frequent occurrence of vascular sclerosis in arteries than veins. This hypothesis needs to be further examined in more mechanistic studies, both in vitro and in vivo.

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