Cardiovascular Pathology 25 (2016) 405–412

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Transmural distribution and connectivity of coronary collaterals within the human heart

Monique G.J.T.B. van Lier [a], Elco Oost [a], Jos A.E. Spaan [a], Pepijn van Horssen [a,1], Allard C. van der Wal [b], Ed vanBavel [a], Maria Siebes [a], Jeroen P.H.M. van den Wijngaard [a,*]

[a] Department of Biomedical Engineering and Physics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands
[b] Department of Pathology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands

ARTICLE INFO

Article history:
Received 24 April 2016
Received in revised form 10 June 2016
Accepted 28 June 2016

Keywords:
Collateral circulation
Imaging
Arteries

ABSTRACT

Despite the importance of collateral vessels in human hearts, a detailed analysis of their distribution within the coronary vasculature based on three-dimensional vascular reconstructions is lacking. This study aimed to classify the transmural distribution and connectivity of coronary collaterals in human hearts. One normotrophic human heart and one hypertrophied human heart with fibrosis in the inferior wall from a previous infarction were obtained. After filling the coronary arteries with fluorescent replica material, hearts were frozen and alternately cut and block-face imaged using an imaging cryomicrotome. Transmural distribution, connectivity, and diameter of collaterals were determined. Numerous collateral vessels were found (normotrophic heart: 12.3 collaterals/cm^3; hypertrophied heart: 3.7 collaterals/cm^3), with 97% and 92%, respectively, of the collaterals located within the perfusion territories (intracoronary collaterals). In the normotrophic heart, intracoronary collaterals {median diameter [interquartile range (IQR)]: 91.4 [73.0–115.7] μm} were most prevalent (74%) within the left anterior descending (LAD) territory. Intercoronary collaterals [median diameter (IQR): 94.3 (79.9–107.4) μm] were almost exclusively (99%) found between the LAD and the left circumflex artery (LCX). In the hypertrophied heart, intracoronary collaterals [median diameter (IQR): 101.1 (84.8–126.0) μm] were located within both the LAD (48%) and LCX (46%) territory. Intercoronary collaterals [median diameter (IQR): 97.8 (89.3–111.2) μm] were most prevalent between the LAD–LCX (68%) and LAD–right coronary artery (28%). This study shows that human hearts have abundant coronary collaterals within all flow territories and layers of the heart. The majority of these collaterals are small intracoronary collaterals, which would have remained undetected by clinical imaging techniques.

1. Introduction

The area of myocardium at risk for infarction may be limited by a well-developed coronary collateral circulation. Coronary collaterals have been identified in healthy human hearts as well as in hearts with coronary artery disease [1–4]. In slowly developing cardiovascular disease, small preexisting collateral arteries may grow by outward remodeling to become connections with higher flow capacity [5,6]. This process, termed *arteriogenesis*, is driven by the increase in fluid shear stress. In addition, new collaterals may develop through angiogenesis, the formation of new vascular segments under the influence of tissue ischemia, and the stimulated release of vascular growth factors [7–9].

Thus far, only limited high-resolution data are available on the complete human coronary tree in three dimensions [10,11]. In one study, postmortem analysis of healthy human coronary vascular corrosion casts shows that collateral lumen diameters range between 20 and 350 μm [2]. A stratification of collaterals into subendocardial,

Abbreviations: IQR, interquartile range; LAD, left anterior descending; LCX, left circumflex artery; LVFW, left ventricular free wall; RCA, right coronary artery; RVW, right ventricular free wall; SEP, septum.

Grants: This work was supported by grants from the European Commission (FP7-ICT-2007/2013 no. 224495: euHeart), the Netherlands Organization for Health Research and Development (ZonMw 91105008), and the Dutch Heart Foundation (DHF 2006B226). This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project EMINENCE (grant 01C-204). J.P.H.M.v.d.W. was funded by a Veni grant from the Netherlands Organization for Scientific Research (NWO/ZonMw 91611171).

* Corresponding author at: Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, L2-28, PO Box 9600, 2300RC Leiden, the Netherlands. Tel.: +31 71 52 99077.
E-mail address: j.p.vandenwijngaard@lumc.nl (J.P.H.M. van den Wijngaard).
[1] Department of Physics and Medical Technology, VU University Medical Center, De Boelelaan 1118, 1081 HZ, Amsterdam, the Netherlands.

http://dx.doi.org/10.1016/j.carpath.2016.06.004

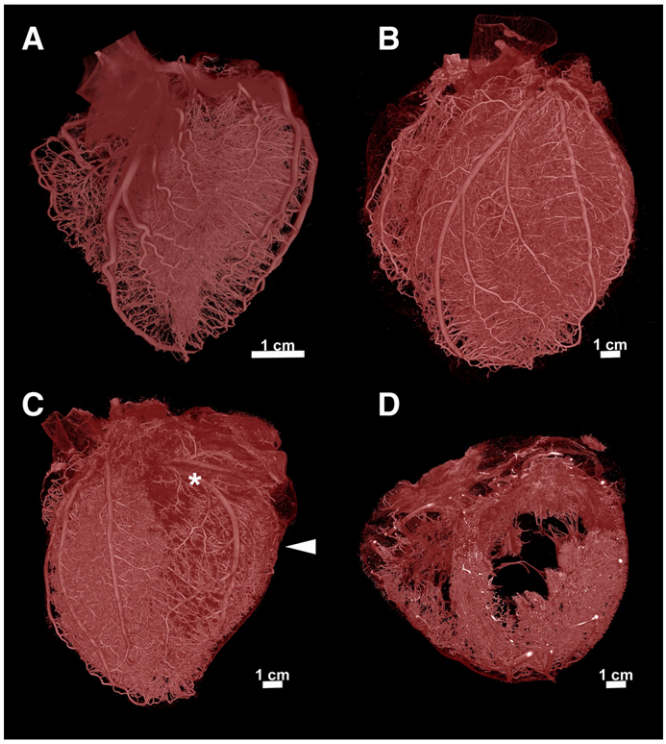

Fig. 1. 3D reconstruction of coronary vascular network in the normotrophic and hypertrophied human heart. (A) Anterior view of the normotrophic heart in 3D. (B) Anterior view of the hypertrophied heart in 3D revealing the entire coronary arterial tree (C) Posterior view of the hypertrophied heart. A decreased vascular density can be observed in LVFW where the left marginal artery runs across the infarcted tissue (asterisk). (D) 3D reconstruction of a transversal slab of the hypertrophied heart of ~2-cm thickness at the level of the arrow head in C.

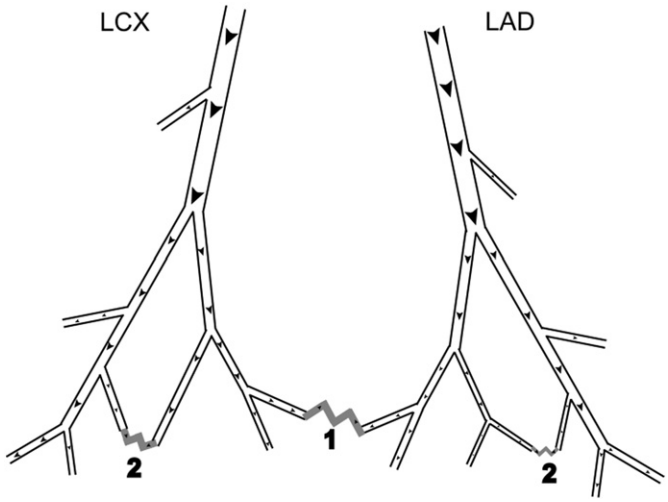

Fig. 2. Definition of collaterals. Starting with seed points at the origin of the major coronary arteries, indicated as LCX and LAD, neighboring points are labeled consecutively down the vascular tree (arrow direction). A segment that was labeled twice identified the presence of a loop in the vascular structure. The segment with the smallest diameter in the loop was assigned as the collateral connection (jagged gray lines). The intercoronary collateral connections are indicated by "1"; intracoronary collaterals are indicated by "2."

midmyocardial, and epicardial layers of the human heart is currently unavailable. In addition, the presence of collaterals within perfusion territories received only little attention until now. Such collaterals may however exert a protective function during local occlusions or rarefaction in the microcirculation and hence prevent the occurrence of focal ischemia or necrosis causing arrhythmia and contractile dysfunction [12,13].

Given the importance of collaterals in the human heart and the scarcity of knowledge about especially the small collaterals, we are working towards a network analysis of coronary flow distribution in man. Such an analysis can be used for assessment of the role of collaterals in protection against acute or chronic local events. As a first step, we performed a detailed analysis of collateral distribution and connectivity based on high-resolution reconstructions of the entire coronary tree.

2. Materials and methods

2.1. Sample preparation

Two hearts were obtained postmortem from the Department of Pathology of the Academic Medical Center (University of Amsterdam). The study met the criteria of the code of good practice for use of human tissue in the Netherlands, and patients' relatives gave written consent.

The first heart (normotrophic) was from an 84-year-old female diagnosed with amyotrophic lateral sclerosis, atrial tachycardia, mitral stenosis, abdominal aortic aneurysm, atherosclerosis, and hypertension. No major cardiovascular events occurred during her lifetime, and the cause of death was noncardiovascular. Postmortem examination yielded a 330-g heart of normal appearance and without congenital anomalies. The second heart (hypertrophied) was from a 64-year-old male diagnosed with amyotrophic lateral sclerosis and exhibiting cardiovascular symptoms including a 27-year-old myocardial infarction, atrial fibrillation treated with a cardiac pacemaker, abdominal aorta prosthesis after a ruptured aneurysm, and popliteal artery stenosis. The residual left ventricular function was 25% of normal. Postmortem examination yielded a severely enlarged heart (weight: 595 g) due to concentric left ventricular hypertrophy and fibrotic scarring of the inferior wall caused by the old infarction. No valvar abnormalities were noticed.

Immediately following autopsy, hearts were suspended from the aorta, and both the right and left main coronary arteries were cannulated. The coronaries were flushed with calcium-free buffer solution until no blood was present in the efflux. The coronary arteries were subsequently filled, at physiological pressure of 80–100 mmHg, with vascular replica material (Batson no. 17, Polysciences, USA) consisting of a monomer base solution, a catalyst, and a promoter. A fluorescent dye, UV-Blue (excitation 375 nm, emission 505 nm, VasQtec, Switzerland), was added to the replica material. During the vascular filling procedure, the ventricular cavities were not pressurized. Instead, the heart was suspended freely by mounting it from the aorta; to avoid stretching, the heart was submerged in calcium-free buffer solution containing adenosine. This setup thereby achieves a geometry permitting most optimal flushing and filling of the vasculature [11]. The vascular replica material was allowed to harden for 24 h at room temperature. Hereafter, the hearts were immersed in carboxymethylcellulose sodium solvent (Brunschwig Chemie, the Netherlands) mixed with 5% Indian ink (Royal Talens, the Netherlands) and frozen at −20°C for at least 24 h.

2.2. Three-dimensional (3D) image acquisition

Image stacks of the entire heart were acquired by using an imaging cryomicrotome. The cryomicrotome setup is described previously [14,15]. In brief, the frozen embedded heart was mounted with its long axis perpendicular to the cutting plane and cut from base to apex. After each cut, the block face of the remaining bulk material was imaged using a 4096×4096, 16-bit resolution digital camera (Apogee Alta U-16, USA) equipped with a variable focus lens (Nikon 70–180 mm, Japan). The normotrophic heart was imaged at 30 μm in-plane resolution; the hypertrophied heart was imaged at 32 μm in-plane resolution due to its larger size. In both cases, slice thickness was identical to the in-plane resolution to achieve isotropic voxel resolution. A reflection