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Anatomy and Physiology: Current Research

Mini Review

Embryological Evidence Disproving Detectability of Blood Vessels Using D2-40

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ABSTRACT

This study [1] aimed to elucidate the venous vasculature in human fetuses with 3D Reconstruction of vessel and nerve localization. Here, it is reported that D2-40 was used as a venous marker, whereas α SMA had been used. D2-40 (PDPN), a lymphatic and mesotheliomal marker, is not expressed in normal blood vessels. It is essential to repeat the study with correct blood vessel specific markers. This paper reports embryological evidence disproving the detectability of blood vessels using D2-40 and provides the correct blood vessel specific markers that should be chosen instead.

KEYWORDS: D2-40 (PDPN), α SMA, Correct Marker Selection, Société Anatomique de Paris

INTRODUCTION

D2-40 is wrong marker to observe venous vessels because D2-40 is the monoclonal antibody to PDPN, a specific marker of lymphatic endothelium. It has physiological function in the separation of lymphatic-venous vessels. In the lymphatic vessel development, lymphatic vessels are separated from venous vessels, and PDPN expression is necessary for the separation. D2-40 (PDPN) is positive only in lymphatic vessels because blood vessels can never express PDPN. On the other hand, there are some pathophysiological cases which D2-40 (PDPN) is expressed in blood vessels in patients with cancer. PDPN also functions as platelet aggrregation-inducing factor, and most cancers highly express PDPN to utilize platelets for own armor against immune systems. If the cancer invasion was occurred in the blood vessels, D2-40 (PDPN) positive cells were shown in the blood vessels. But in this study, the normal fetuses were used as samples, so it can not be considered that D2-40 (PDPN) positive cells are shown in venous vessels. Taking into consideration the factors mentioned above, there are serious contradictions because it is impossible to reconstruct 3D venous system with D2-40 (PDPN).

TEXT

THE CORRECT MARKER SELECTION for DISTINGUISHING BLOOD and LYMPHATIC VESSELS

It is important to perform experiments with accurate identification of venous vessels, and to prevent mistakes due to the wrong marker selection. Here I present Vessel specific markers. Various blood vessel markers are known , CD31, CD34, vWF, α SMA, etc. included. However, most markers well used as the blood vessel markers are often expressed widely also on the lymphatic vessels. Thus, it is important to verify the markers which are truly distinguishable blood vessels, except for lymphatic vessels. In order to select the high specific markers, I organize the function and the specificity of each marker protein.

CD31(PECAM-1) (Blood vessel +/ Lymphatic vessel +)

[F] Function: *Endothelial cell intracellular adhesion, platelet aggregation, and leukocyte transendothelial migration*

CD31 has an essential role for lumen formation in endothelial cells via "Cord-Hollowing Mechanism" [2, 3]. Therefore CD31 is well used as endothelial, but one should pay attention to use this antigen as a blood specific marker. CD31 should be used as a pan-endothelial marker because it is expressed not only in blood

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vessels, but also in lymphatic vessels to play role for lumen formation process in both vessels [4].

CD34 (Blood vessel + / Lymphatic vessel ±)

[F]: *Endothelial cell intracellular adhesion and leukocyte transendothelial migration*

CD34 is also expressed in endothelial cells and plays an essential role in lumen formation via "Cord-Hollowing Mechanism". This antigen is expressed also in both blood and lymphatic vessels. However, CD34 expressions are less or none in lymphatic vessels than blood vessels. This fact can show that CD34 has usability as a blood vessel marker depending on the tissues, but in another word, CD 34 does not enough detectability to distinguish the blood and lymphatic vessels completely. It is required to select other markers when complete specificity is required.

vWF (Blood vessel + / Lymphatic vessel ±)

[F]: *Hemostasis via binding to factor VIII*

vWF has an essential role in coagulation and often in thrombosis because of the plaque formation in blood vessels. vWF is regarded as a blood vessel marker because its functions related to the blood vessels deeply, but weak vWF expression can be observed also in lymphatic vessels depending on the tissues [5]. vWF should be used as a pan-endothelial marker because it can be expressed in both blood and lymphatic vessels.

α SMA (ACTA2) (Blood vessel + / Lymphatic vessel– (Collecting duct +))

[F]: *Muscle smooth cell contraction*

α SMA is expressed in the SMCs. α SMA is highly expressed in all blood vessels because blood vessels including both arterial and venous vessels are surrounded by SMCs. For this reason, α SMA is well used as a blood vessel marker. However, α SMA can be used as a blood vessel marker only when only capillary lymphatic vessels are included the samples because collecting lymphatic vessels are surrounded by lymphatic smooth muscle cells expressing α SMA [6]. In these collecting vessels, α SMA expression can be highly detected also in the lymphatic vessels as well as the blood vessels [7].

VEGFR-1 (Flt-1) (Blood vessel + / Lymphatic vessel +)

[F]: *Induction of blood vessel formation*

VEGFR-1, a receptor of VEGF-A, -B, -F, and Placenta Growth Factor (PGF), plays an important role as key component for angiogenesis and lymphangiogenesis [8, 9]. Although the function is thought to suppress the VEGFR-2-induced exceed angiogenesis to prevent forming the abnormal blood vessels rather than to stimulate angiogenesis in the embryonic vasculogenesis [10]. VEGFR-1 is expressed in both blood and lymphatic vessels [11], and therefore VEGFR-1 can be a blood vessel specific marker showing no positive in lymphatic vessels.

VEGFR-2 (FLK-1, KDR) (Blood vessel + / Lymphatic vessel +)

[F]: *Induction of blood and lymphatic vessel formation*

VEGFR-2 has strong functions in the angiogenesis via binding with VEGF-A, -E, and -F and lymphangiogenesis via binding with VEGF-C and –D [12]. VEGFR-2 is expressed in both blood and lymphatic vessels, and therefore VEGFR-2 can not be used for the specific marker to distinguish between blood and lymphatic vessels.

VEGFR-3 (FLT-4) (Blood vessel +/ Lymphatic vessel +)

[F]: *Induction of lymphatic vessel formation*

VEGFR-3 has an important function in the lymphangiogenesis via binding its ligands VEGF-C and –D [12]. VEGFR-3 is expressed only in lymphatic vessels in normal tissues, but in cancer tissues, VEGFR-3 is expressed in blood vessels weakly not only in lymphatic vessels because cancer-induced lymphangiogenesis is occurred to the metastasis [13].

Eph B4 (Venous vessel + / Lymphatic vessel ±)
Ephlin B2 (Arterial vessel+/Lymphatic vessel ±)

[F]: *Segrgation of artery and vein*

Eph B4 and Ephlin B2 have essential roles for vasculogenesis [14]. Tyrosine kinase receptor Eph B4 is expressed in venous endothelial cells and its transmembrane ligand Ephlin B2 is expressed in arterial endothelial cells specifically, and these expressions decide differentiation of venous, arterial vessels and Eph/Ephlin interaction mediates repulsion and disengagement to form correct position between each vessel [15]. However, Eph B4 and Ephrin B2 have also an essential role for lymphatic valve development and be expressed in lymphatic vessels [16, 17, 18]. Therefore, Eph B4 and Ephlin B2 are useful to distinguish the venous and arterial vessels, but they can not be used as blood vessel specific marker to distinguish the blood vessel from lymphatic vessels.

APJ (Venous vessel + / Lymphatic vessel ±)
Apelin (Arterial vessel +/ Lymphatic vessel ±)

[F]: *Alignment of artery and vein*

As well as Eph/Ephlin system, APJ/Apelin system also plays essential roles for vasculogenesis and lymphangiogenesis. These proteins mediate attraction between venous and arterial vessel in contrast to Eph/Ephlin system. The correct position between venous and arterial vessels are regulated by cooperation between APJ/Apelin and Eph/Ephlin system. It is said that G protein-coupled receptor APJ is expressed in venous endothelial cells and its ligand Apelin is expressed in arterial endothelial cells. However, APJ and Apelin are expressed in various tissues widely rather than venous/arterial vessels specific expression [19]. It is not suitable for use of these proteins as specific markers to distinguish blood/lymphatic vessels.

LYVE-1 (Blood vessel - / Lymphatic vessel +)

[F]: *Promoting lymphvasculogenesis in the venous vessel*

LYVE-1 has an essential role for early lymphvasculogenesis through mediating the expression of SOX18 in the subpopulations of venous endothelial cells, results in stimulating differentiation potency into the lymphatic endothelial cells. LYVE-1 is not expressed in blood vessels, therefore this protein can act as a kind of strongest marker for lymphatic vessels including the lymphatic endothelial precursor cells in the venous vessels [20]. But one should pay attention to detect the collecting lymphatic vessels because LYVE-1 is attenuated to negative expression in the collecting vessels in contrast to the positive expression in the capillaries [21].

Prox-1 (Blood vessel - / Lymphatic vessel +)

[F]: *Promoting lymphvasculogenesis in venous vessel*

Prox-1 has an essential role for differentiation from LYVE-1-expressed lymphatic endothelial precursor cells into lymphatic endothelial cells. Prox-1 expression is stimulated by cooperation between SOX-18 and COUP-TFII. Prox-1 is expressed highly in lymphatic endothelial cells but not in blood vessels, therefore Prox-1 can be used as one of the strongest lymphatic vessel specific markers [22].

PDPN (Blood vessel ± / Lymphatic vessel +)

[F]: *separation of lymphatic vessel from venous vessel*

PDPN functions as a platelet-aggregation inducing factor and has an essential role for the final stage of lymphvasculogenesis via mediating separation of the lymphatic endothelial cells from the venous vessel. In normal tissues, PDPN is highly expressed in lymphatic vessels but no expressions are observed in blood vessels, therefore PDPN can be used as one of the strongest lymphatic vessel specific markers. However, one should pay attention in pathological tissues because the metastasis tumor expresses PDPN to aggregate platelet surrounding itself to prevent the attack from the immune system, so PDPN expressions can be detected in the wide tissues [23]. Or in another case, inflammatory disease skin such as eczema or psoriasis expresses PDPN also in blood vessels [24]. As mentioned above, PDPN is expressed in these pathological tissues besides lymphatic vessels, therefore PDPN can be used as a lymphatic vessel specific marker only in normal tissues.

For LYVE-1, Prox-1, PDPN , the word "lymphvasculogenesis": [25] (sprouting from the vein), was intentionally used here.

Société Anatomique de Paris (Program and PPT), 29 juin 2012

SOCIÉTÉ ANATOMIQUE 12 FRIMAIRE AN 12.

SOCIETE ANATOMIQUE DE PARIS

45 rue des Saints-Pères 75270 PARIS CEDEX 06

Séance du Vendredi 29 juin 2012
à 17 heures
Salle LAVOISIER A
Les communications sont de 10 minutes
suivies de 10 minutes de discussion

5-Noriko KUROBE, Jean-François UHL

Université Paris Descartes, URDIA, EA4465, Anatomie

Recontruction 3D des veines des membres inférieurs d'un fœtus de 14 semaines

3 D reconstruction of the veins of the lower limb of a fetus of 14 weeks

Objectifs : L'organogénèse humaine du système veineux des membres inférieurs est mal connue et aucune observation n'est disponible. Cette étude réalise la modélisation du système veineux des membres inférieurs d'un fœtus humain de 14 semaines.

Matériel et méthodes : la technique de la dissection anatomique assistée par ordinateur (DAAO)[1] a été utilisée. Après avoir inclus les deux membres inférieurs d'un fœtus de 14 semaines en paraffine, des coupes de 5mm d'épaisseur ont été faites, et divisées en 10 blocs. Les quatre premières coupes de chaque bloc ont été étudiées par les 4 colorations et immuno-marquages différents ci-dessous.

-Hématoxyline éosine safran (HES) : considéré comme la coupe de référence

-Trichrome de Masson pour identifier les fibres de collagènes colorées en bleu par l'aniline.

-La protéine S100 est un immuno-marquage pour les nerfs

-Le D2-40 a été utilisé comme un immuno-marqueur du système vasculaire.

La numérisation des coupes colorées (800 images) a été faite avec un scanner à 600 DPI. Apres recalage et numérotation des coupes, la technique de reconstruction 3D a été réalisée par segmentation manuelle en utilisant le logiciel Winsurf version 3.5 pour obtenir un modèle vectoriel des structures d'intérêt : peau, os, muscles, nerfs, artères et veines.

Résultats : Nous avons trouvé une grosse veine axiale des deux cotés accompagnant le nerf sciatique, suggérant que c'est le vaisseau principal de la cuisse à la fin de l'organogénèse. Cette veine hypoplasique chez l'adulte, se réduisant à une petite arche dans 95 % des cas [2]

Conclusion : La technique de DAAO est la seule capable de produire un tel modèle 3d du système vasculaire et nerveux. Ces résultats confirment la théorie des nerfs « angio-directeurs » : l'embryogénèse des veines suit le développement des nerfs[3]. Nous avons en effet observé la relation intime entre les veines principales et les nerfs confirmant le rôle important du facteur de croissance de l'endothélium vasculaire (VEGF) sécrété par les nerfs. Il stimule la maturation des vaisseaux le long des nerfs, et induit leur spécialisation en artères, veines ou lymphatiques.

Mots clés : fœtus, organogénèse, nerfs pelviens, modélisation, Computer Assisted Anatomical Dissection

1) Alsaid B et al. Computer assisted anatomic dissection , evolution , methodology and application in intra-pelvic innervation study. SRA, 2011, 33, 5, 397-404

2) Uhl J.F. Gillot C. Chahim M. The anatomical variations of the femoral vein. Journal of vascular surgery, 2010, 52, 714-719

3) Uhl, JF, Gillot C. Embryology and 3D anatomy of the venous system of the lower limbs. Phlebology, 2007, 22, 5, 194-20

Société anatomique de Paris du 29 juin 2012

Figure 1: Program (J.F.Uhl, 2012)

Figure 2(a): PPT (J.F. Uhl, 2012)

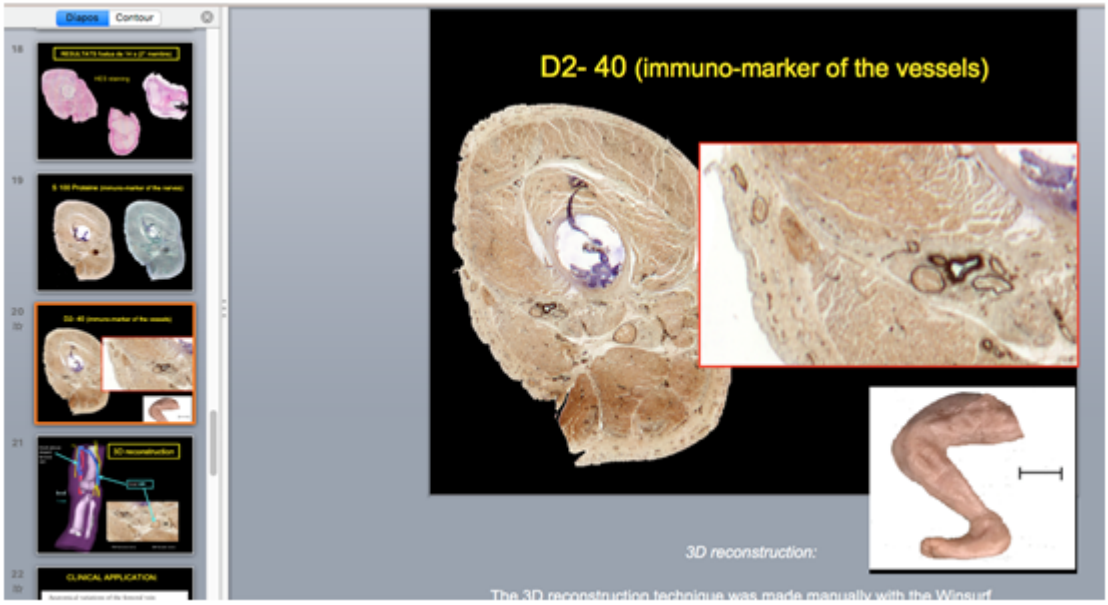


Figure 2(b): PPT (J.F.Uhl , 2012)

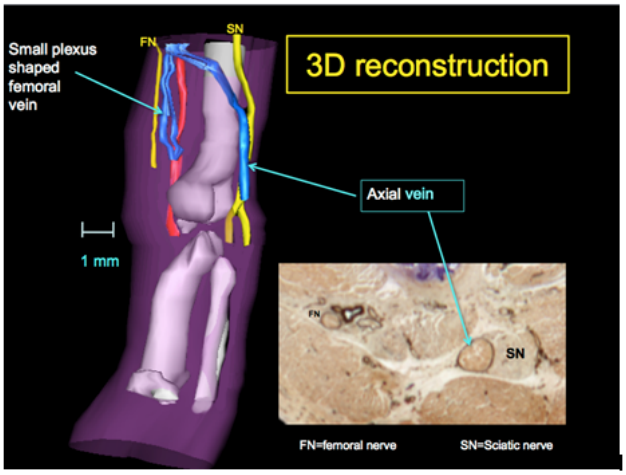
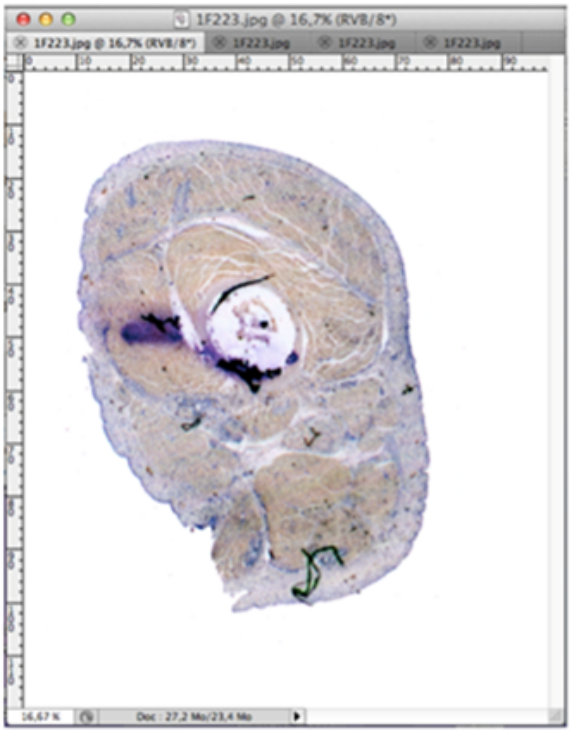
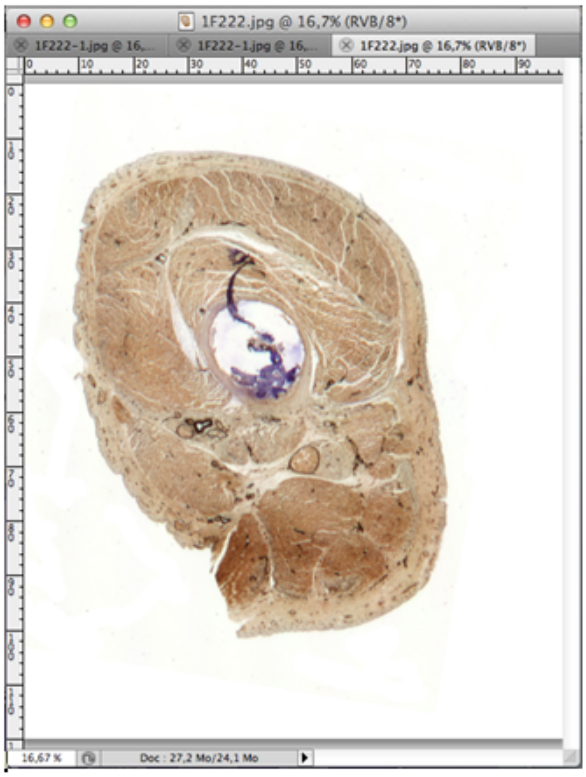


Figure 2(c): PPT (J.F. Uhl , 2012)

PPT as well as Program was made by J.F. Uhl, without the consent or knowledge of Kurobe and Claude Gillot. Kurobe rejected the presentation because of the wrong methodology. Because of the absence of Uhl without notice, on that day, Kurobe was forced to make a speech and began to explain his wrong IHC marker selection, when Prof. Vincent Delmas (Director of the fac), cried « Stop.That's all. »



CF : α SMA, 1F222 (Kurobe, 2011)



D2-40, 1F223 (Kurobe, 2011)

Claude Gillot

He was only Prof. who could understand the wrong Methodology of Uhl. He said. « Uhl made a serious mistake. There is responsability on the side of Univ. » (09.2015). He decided to become Supervisor of Kurobe and ordered to submit one article on Hunter's Canal(HC) to J. Phlebology. He provided over 50 photos : Cross-Section through the middle third of the thigh ; human adult, for that. His lecture was suddenly discontinued because of his open-heart operation.

Prof. Claude Gillot: ex-President of Société Anatomique de Paris

Directeur de Dissection

Centre du Don des Corps, 6ème étage

45 rue des Saints-Pères

75006 Paris

Personal instruction in HC: officially recognized by the faculty of Anatomy

09.2015-03.2016, (16 :00-18 :00) every Friday

CONCLUSION

D2-40 can not be used to detect the venous vessels. This study contains a significant error in methodology. I claim that the correct methodology to detect the venous vessels, should be performed with the correct marker combination which include the pan-endothelial marker e.g. CD31, lymphatic vessel marker e.g. LYVE-1, Prox-1, or PDPN, and venous vessel marker e.g. Eph B4. In this combination, the venous vessels can be detected, as $CD31^{+}$ / $LYVE\text{-}1^{-}$, $Prox\text{-}1^{-}$, or $PDPN^{-}$ / $Eph\ B4^{+}$. In many

journals [26, 27, 28, 29] , J.F. Uhl introduces this study « Figure 2 (a) (b) (c) », with the expression of α SMA :1F222. This is not the first report which observed the venous system of normal human fetus with D2-40. I request J.F.Uhl to retract these paper immediately and present the venous system accurately after performing the experiments again using an accurate marker specific to venous vessels. D2-40 is inappropriate for 3D Reconstruction and observation of the venous system.

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