Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists

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Edited by Robert Fitridge and Matthew Thompson

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Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotropic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α-amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β2-GPI	β2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonic gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL1-β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC_{20}	Myosin light $chain_{20}$
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI_2	Prostacyclin
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
$PGEI_2/PGI_2$	Prostaglandin I_2
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca^{2+} APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
TαCE	TNFα converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

27 • Lymphoedema – Principles, Genetics and Pathophysiology

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INTRODUCTION

The lymphatic circulation consists of a network of blind-ended capillaries lined with endothelial cells that drain into larger vascular trunks and eventually empty into the blood circulation. It is otherwise totally separate from the blood circulation although lymphatics are often anatomically related to arteries and veins. Lymphatic vessels are found in nearly all tissues and have several important functions including transportation of fluids, plasma macromolecules, and cells back to the blood circulation. The lymphatics also form a major transport route for lipids absorbed from the intestinal tract, and are a critical component of the immune system transporting leucocytes and antigens from the tissues to the lymphoid organs.

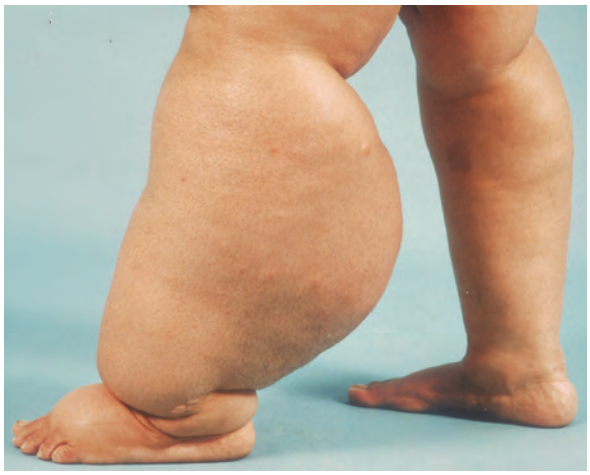
Lymphoedema is an accumulation of tissue fluid in the interstitial space as a result of failure of the lymphatic circulation. This can be severe and disfiguring. Defects in the lymphatic system can be primary or acquired. Lymphoedema most frequently affects the legs (80%) although can present as swelling of the arms, face or external genitalia. (Figure 27.1)

CLASSIFICATION OF LYMPHOEDEMA

The diagnosis of lymphoedema should be reserved for those patients in whom a secondary cause of oedematous swelling has been excluded. (Table 27.1). Chronic venous disease is a common cause of unilateral swelling and there are often other characteristic skin changes. Sub-clinical lymphoedema sometimes becomes apparent when other conditions such as venous hypertension cause an increase in fluid and protein forced into the interstitial space that overwhelms a poorly functioning lymphatic system.

Lymphoedema is classified as primary when there is an intrinsic defect in the lymphatic vessels or nodes that leads to failure to drain lymph from the tissues. It has an incidence of 1:6000 and is three times more common in women than men.[1]

Secondary lymphoedema is more common and occurs when the lymphatics are damaged by a defined external cause. The commonest cause worldwide with approximately 120 million cases is filarial infection (*Wuchereria bancrofti, Brugia malayi*



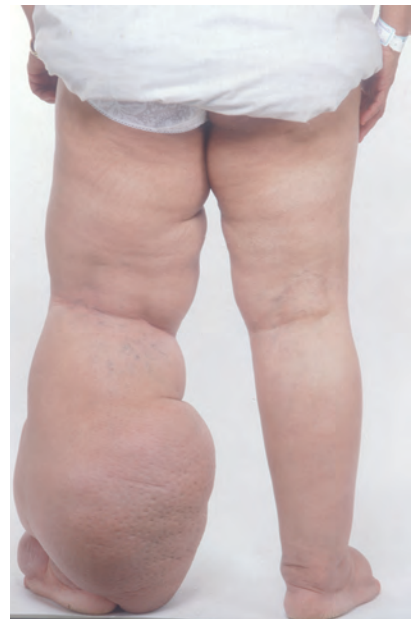


FIGURE 27.1 (a,b): Lower limb lymphoedema

or Brugia timori) leading to inflammation and fibrosis of lymph nodes or the adjacent lymphatics.[2] This often presents as hydrocele (in men), massive lymphoedema and elephantiasis. It is common in tropical and sub-tropical regions of Africa, the Far East and South America. Another common cause in the tropics is podoconiosis (endemic non-filarial elephantiasis), a geochemical disease that occurs in individuals exposed to red clay soil derived from alkalic volcanic rock.[3] Ultra fine silica particles are absorbed through the skin of barefoot agricultural workers and cause a progressive obliterative lymphangitis.

In Europe and North America secondary lymphoedema is usually related to trauma, surgery, and radiotherapy, often associated with the treatment of malignancy.

Classification of primary lymphoedema

The original classification of primary lymphoedema according to age of onset into congenital (present at birth), praecox (appearing before 35 years of age) and tarda is of little use in differentiating the underlying disease processes. In the 1950s Kinmonth proposed both the clinical distinction of primary and secondary lymphoedema and a classification system based on lymphangiographic appearance.[4] Browse later combined these into a system that reflected clinical and aetiological factors known at the time.[5]

1) Primary lymphoedema: Lymphoedema caused by a primary abnormality or disease of the lymph conducting elements of the lymph vessels or lymph nodes. Those in which the functional abnormality and its cause are known are divided into three groups:
   a. large vessel abnormalities such as congenital aplasia of the thoracic duct or cysterna chyli
   b. congenital lymphatic valvular incompetence or congenital aplasia
   c. lymph node fibrosis.

TABLE 27.1:

Secondary causes of swelling that must be excluded before making a diagnosis of lymphoedema
Cardiac failure
Renal failure
Hepatic failure
Hypoproteinaemia
Allergic disorders
Vasculitis
Hereditary angio-oedema
Idiopathic cyclic oedema
Venous insufficiency (Obstruction or reflux)
Vascular malformations
Lipoedema / lipodystrophy
Functional (disuse)
Factitious
Gigantism (overgrowth syndromes)
Investigations to exclude other causes of swelling
ECG
Echocardiography
FBC
U+E / Creatinine
LFT including albumin
CRP / ESR
Autoimmune screen
Complement tests
Venous duplex
Contrast venography
MRI for soft tissue swelling / vascular malformation
CT abdomen and pelvis
Lymphoscintigraphy
Lymphography

The remainder are characterised by a reduced number of lymphatics on lymphography

2) Secondary lymphoedema: Oedema caused by disease in the nodes or vessels that began elsewhere (e.g., neoplasia or filariasis), or lymphocytic proliferative disorders such as Hodgkin's disease or following surgical extirpation of lymph nodes or vessels.

More recently genetic abnormalities have been discovered in both congenital (present at birth) and delayed onset forms of lymphoedema and this has lead to a modified view of this classification. There is also a distinction between 'lymphangio-obstructive' and 'lymphangio-obliterative' to indicate underlying pathology.

1) Genetically determined abnormalities
   a. Aplasia, malformation and valvular incompetence of the central lymphatic ducts, namely the cisterna chyli and thoracic duct
   b. Aplasia, hypoplasia or dilatation and valvular incompetence of the collecting ducts in the subcutaneous tissues of the limb and trunk. This group therefore includes the familial conditions such as Milroy's, Meige's and lymphoedema-distichiasis syndromes. This group also includes sporadic congenital lymphoedema associated with some recognised congenital abnormalities. (Table 27.2).
2) Acquired abnormalities
   a. Lymphangio-obliterative lymphoedema
      i. Distal
      ii. Proximal
      iii. Combined
   b. Intra-glandular (hilar) fibrosis; representing the lymphangio-obliterative process in the lymph conducting parts of the lymph gland

3) Kinmonth's numerical hyperplasia; the lymphangiographical abnormality is of increased numbers of normally sized lymphatic channels associated with excessive numbers of small lymph glands.

THE GENETICS OF LYMPHANGIOGENESIS IN PRIMARY LYMPHOEDEMA

Milroy's disease

Milroy first described a syndrome of inherited, painless, non-progressive swelling of the legs present at birth in 1892.[6] The family genealogy of the affected clergyman was followed across six generations and 22 out of 97 descendants were thought to have limb swelling indicative of lymphoedema.

Milroy's disease is an autosomal dominantly inherited condition. Linkage studies have mapped the condition to a locus on chromosome 5q35.3. More than 30 mutations in vascular endothelial growth factor receptor-3 (VEGFR-3), which maps to this region, have now been identified.[7–13] De novo mutations in the VEGFR-3 have also now been reported in patients with sporadic congenital lymphoedema.[14,15]

VEGFR-3 is the receptor for VEGF-C and VEGF-D. VEGF-C acts through VEGFR-2 and VEGFR-3 during formation of the vascular system, with expression of VEGFR-3 becoming restricted to the lymphatic endothelium.[16,17,18] The ability to specifically target lymphatic endothelium has allowed the visualisation of channels in mouse and human lymphatics with markers such as lymphatic vessel endothelial hyaluronan receptor (LYVE-1)[19] (Figure 27.2).

TABLE 27.2:

Disorders and syndromes involving primary lymphoedema
Milroy disease
Lymphoedema-distichiasis syndrome
Hypotrichosis-lymphoedema-telangiectasia syndrome
Meige disease (Primary non-syndromic lymphoedema)
Lymphoedema and yellow nails
Lymphoedema with ptosis
Hennekam syndrome (Lymphoedema-lymphangiectasia-mental retardation)
Aagenaes syndrome (Hereditary intrahepatic cholestasis with lymphoedema)
Microcephaly-lymphoedema-chorioretinal dysplasia (MLCD)
Noonan syndrome
Turner syndrome (45, X karyotype)
Prader-Willi syndrome
Klippel-Trenaunay syndrome
Maffucci syndrome
Proteus syndrome

Transfection of adenoviral VEGF-C into the skin of mice causes massive dermal lymphangiogenesis,[20,21,22] and transgenic expression of VEGF-C in mice increases lymphatic endothelial cell proliferation and causes lymphatic channel hyperplasia.[23] In contrast, targeted deletion of VEGFR-3 in mice causes defective vasculogenesis and embryonic death.[24] Transgenic mice expressing a soluble form of VEGFR-3 that is a potent inhibitor of VEGF-C and –D signalling survive into adulthood if a keratinocyte promoter is used to deliver the genetic mutation selectively to the dermis.[25] These animals have a normal blood vasculature but develop a lymphoedema phenotype with swollen limbs. These studies show that VEGFR-3 has an essential role in lymphangiogenesis. Further study of patients with Milroy's disease show that the lymphatics in the upper limb are completely normal, and in the lower limb they are present in the skin but there is no functional uptake.[13]

Lymphoedema-distichiasis syndrome

Lymphoedema-distichiasis syndrome is an autosomal dominantly inherited condition caused by mutations in the FOXC2 (forkhead transcription factor) gene at 16q24 locus.[26] Distichiasis described an extra growth of eyelashes from the Meibomian glands, and 30% also have ptosis. Distichiasis often causes corneal irritation, recurrent conjunctivitis and photophobia (Figure 27.3). It can be treated in a number a ways, including lubrication, plucking, electrolysis and surgery. The condition is associated with other congenital abnormalities including congenital heart defects (tetralogy of Fallot), cleft lip and palate, varicose veins and spinal extradural cysts.[27]

In this condition distichiasis is present from birth and lymphoedema appears from puberty. It is often bilateral and is usually below the knee. Duplex ultrasound and lymphoscintigraphy reveal that patients have both lymph and venous reflux in lower

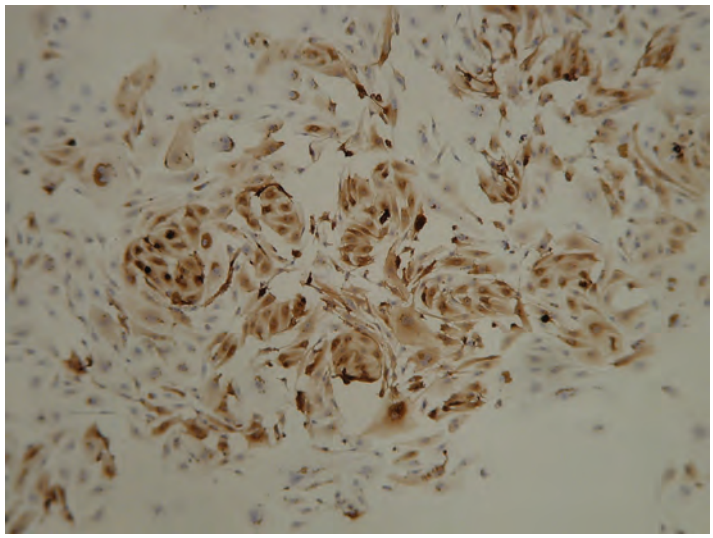


FIGURE 27.2: Human dermal tissue stained with marker for LYVE-1 expression on lymphatic endothelium

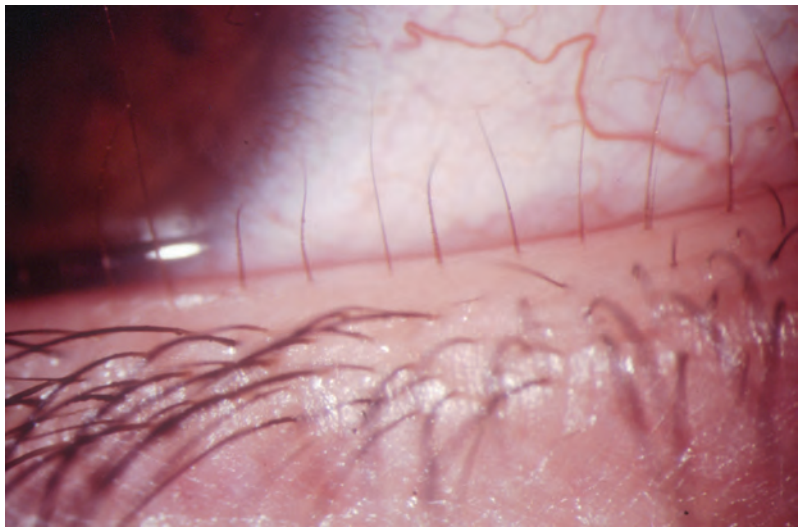


FIGURE 27.3: Distichiasis with accessory eyelashes along the posterior border of the lid margin in the position of the Meibomian glands

limbs, suggesting primary valve failure.[28] Skin biopsies in individuals with FOXC2 mutations demonstrate an abnormally large proportion of lymphatic vessels which are covered with smooth muscle cells, compared to family members without the mutation. Similar findings in FOXC2 knockout mice indicates that FOXC2 is both essential for valve morphogenesis as well as normal interactions between lymphatic endothelial cells and pericytes.[29] Venous reflux in lymphoedema-distichiasis syndrome could be a significant factor in the onset and progression of swelling.

Hypotrichosis-lymphoedema-telangiectasia syndrome

Hypotrichosis-lymphedema-telangiectasia syndrome is caused by mutations in the transcription factor gene SOX18.[30] This extremely rare syndrome is characterized by the association of childhood-onset lymphoedema in the legs, loss of hair, and telangiectasia, particularly in the palms. Inheritance is either autosomal dominant or autosomal recessive. Studies of the naturally occurring SOX18-mutant mouse strain suggest abnormal expression of a number of downstream gene targets required for structural integrity during microvascular maturation.[31] SOX18 directly activates transcription of the Prox1 gene which controls lymphatic vessel development from endothelial precursor cells.[32]

Meige disease (primary non-syndromic lymphoedema)

In 1898, Henri Meige described the most common variety of primary lymphoedema.[33] Meige disease is a familial lymphoedema developing at or soon after puberty in which no other congenital abnormality is identified. The lymphoedema is often symmetrical, rarely extends above the knee, and is clinically indistinguishable from that found in lymphoedema-distichiasis syndrome. It occurs three times more commonly in females than males and has a genetic predisposition in about a third of cases. Lymphography demonstrates peripheral

lymphatic hypoplasia with more proximal lymphatic channels remaining patent. The genetic abnormality in this syndrome has not been discovered but it has been shown not to involve the FOXC2 gene.[34]

Other primary lymphoedema disorders

Two other very rare forms of primary lymphoedema have been proposed to exist; lymphoedema associated with discoloured, slow growing and excessively curved nails (lymphoedema and yellow nail syndrome), and lymphoedema with ptosis. These may both represent poorly phenotyped cases rather than truly exist as separate entities, as yellow nails can be found in Milroy's disease, Meige disease and lymphoedema-distichiasis, and ptosis occurs in lymphoedema-distichiasis.[33]

Many other syndromes are known to have lymphoedema as a clinical feature (Table 27.2). Lymphoedema may affect the whole body or can affect arms, legs, face, conjunctiva, and the genitalia in a segmental pattern. In primary lymphoedema, facial, conjunctival or genital lymphoedema is often associated with limb involvement. Systemic disorders of the lymphatics include intestinal lymphangiectasia, chylous ascites, pleural effusions, pericardial effusions and pulmonary lymphangiectasia. The surgical treatment of these disorders is complex and may in involve ligation or excision of refluxing lymphatics, or drainage procedures.

STRUCTURE AND DEVELOPMENT OF THE LYMPHATIC CIRCULATION

The lymphatic system consists of a vascular network of thin-walled, blind ended capillaries made up of a single-cell layer of endothelial cells joined by discontinuous button-like junctions that open in response to increased interstitial fluid pressure.[35] Lymphatic capillaries have no basement membrane or supporting smooth muscle cells or pericytes, and so are highly permeable to protein-rich lymph fluid. They do, however, possess specialised anchoring filaments that link the endothelial cells to surrounding matrix and tissues; these help keep the capillaries open and increase their permeability as interstitial pressure rises.[36-38] The lymphatic capillaries converge into precollecting lymphatic vessels and these carry lymph to the main collecting trunks (e.g. the thoracic duct) for return to the venous circulation. Unlike lymphatic capillaries, precollecting and collecting trunks contain smooth muscle cells and pericytes. Collecting lymphatics also have internal valves to prevent retrograde flow of lymph fluid.

Early research into the origin of the lymphatics relied on either injection of substances (dyes or resins) into the circulation or serial sectioning to visualise early lymphatic vessel and sac development. This resulted in two opposing models: the first proposed by anatomists and embryologists using injection techniques that the lymphatic vessels bud off the primitive veins and grow out by lymphangiogenesis;[39] and the second that lymphatic vessels arise from the mesenchymal spaces with lymphatic sacs coalescing to form vessels.[40]

More recently molecular techniques have better characterised the origin of lymphatics in several models.[41] Studies using VEGFR-3 expression as a marker of lymphatic endothelial cells (LECs) in an avian model have suggested a dual origin from mesodermal lymphangioblasts and adjacent veins[42] and similar conclusions have been drawn in an amphibian model examining staged expression of the prospero-related homeobox gene, Prox1.[43] A number of other studies, both in mice and a zebrafish model, have concluded

that the majority of cells contributing to LEC arise from primitive veins. If there is a haematopoietic contribution to LEC this occurs relatively late and peripherally in their development, and may also contribute to postnatal physiological or pathological lymphangiogenesis.

The homeobox transcription factor, Prox1 is required for lymphatic cell differentiation. Prox1 is exclusively expressed in a subpopulation of endothelial cells in the anterior cardinal vein that emerge from the vein and form lymph sacs.[44] Prox1 knockout mouse embryos do not develop lymphatic vessels[44] with the budding embryonic venous endothelial cells defaulting to a blood vascular rather than lymphatic phenotype.[45] Lineage tracing studies have provided further evidence that LECs sprout, proliferate and migrate from venous-derived lymph sacs and haematopoietic cells do not contribute to the mammalian lymphatic system.[46] Temporal inactivation of Prox1 during embryonic and postnatal lymphangiogenesis causes loss of LEC phenotype and reversion to a blood vessel endothelial phenotype.[47]

Recent studies suggest that the transcription factor SOX18 controls Prox1 expression. Mutations in SOX18 were identified in patients with hypotrichosis-lymphoedema-telangiectasia syndrome.[30] SOX18 directly controls Prox1 expression by binding to its promoter; SOX18 knockout mice do not express Prox1 in cardinal vein endothelial cells and develop gross oedema.[32]

Further proliferation and migration of LECs from embryonic veins is controlled by VEGFR-3. Initially expressed in both blood and LECs, expression becomes restricted during embryogenesis. As discussed above, mutations in VEGFR-3 cause Milroy's disease in humans. VEGFC is the principal ligand of VEGFR-3; in VEGFC knockout mice LECS are correctly specified, as defined by the normal expression of LYVE-1, Prox1 and VEGFR-3, but fail to proliferate and migrate.[21] Neuropilin-2 is a non-signalling transmembrane receptor that acts as a co-receptor for VEGFR-3; Neuropilin-2 knockout mice have lymphatic hypoplasia with normal development of arterial and venous vasculature.[48] The transcription factor Tbx1 activates VEGFR-3 expression in endothelial cells and is the major gene for DiGeorge syndrome in humans. Tbx1 does not seem to be required for LEC differentiation but is needed for further growth and maintenance of lymphatic vessels; deletions in the gene cause widespread disruption of lymphangiogenesis.[49]

A number of other genes have been implicated in further lymphatic maturation and remodelling. Mutations in the forkhead transcription factor FOXC2 have been found in patients with lymphoedema-distichiasis syndrome, as discussed above. Lymphatic vessels are correctly differentiated in FOXC2 knockout mice[50] but there is abnormal recruitment of smooth muscle cells to lymphatic capillaries as well as agenesis of lymphatic valves.[29] Recently FOXC2 has been shown to play an important role in the formation of mature lymphatic collectors, including the formation of valves, recruitment of mural cells and smooth muscle, and pruning of branches.[51] In addition the transcription factor NFATc1 interacts with FOXC2 binding enhancers during valve formation.

Many other genes that have been implicated in abnormal lymphatic maturation including Angiopoietin-2,[52] EphrinB2, Aspp51, Emilin1, Slp76 and Syk.[41] For example Ang-2 knockout mice have subcutaneous oedema and chylous ascites; their lymphatics have a disorganised appearance, with poorly developed and disorganised circumferential smooth muscle coat. The relationship between these genes and human conditions has yet to be defined. Platelets may also play

a role in separating blood and lymphatic vessels during embryogenesis.[53] Understanding the mechanisms of lymphangiogenesis may lead to pro-lymphangiogenic treatments for lymphoedema.[54]

CLINICAL ASPECTS OF LYMPHOEDEMA

Most patients present with unilateral leg swelling. At an early stage the swelling will easily pit if pressure is applied, but chronic lymphoedema is associated with fibrosis and the subcutaneous tissues become thickened and hard. At a microscopic level the perilymphatic space becomes chronically thickened with a granulofilamentous material containing degenerate elastic fibres and collagen. In the presence of poorly functioning lymphatics, interstitial fluid becomes stagnant and can become infected; sometimes an infection may be an initial event and the subsequent swelling is blamed on this, but it is more likely to be a consequence of pre-existing abnormal lymphatic drainage. *Streptococcus pyogenes* is the most common pathogen. Patients may present with recurrent episodes of cellulitis, and each episode of infection predisposes to fibrosis and further lymphatic damage. Acute inflammation induces hyperaemia and increased hydrostatic pressure as well as increased vascular permeability and so increases accumulation of protein rich interstitial fluid. Endothelial derived nitrous oxide and oxygen free radicals are released; these are vasodilators and also inhibit the spontaneous tonic and phasic contractions of the lymphatic vessel wall smooth muscle, further reducing lymphatic flow.

Inguinal lymph nodes may be enlarged, especially if there is pelvic obstruction. Cutaneous lymphatic vesicles or a capillary naevus are signs of underlying megalymphatics with reflux. Release of cytokines causes thickening of dermal keratinocytes and an acanthotic appearance of the dermis. Inflammatory cells migrate from the papillary dermal layers into the epidermal cell layer. Microfilament deposition in the dermo-epidermal junction leads to a thickened epidermal basal lamina. This hyperplasia and hypertrophy of the dermal vascular endothelial and epidermal cells is responsible for the abnormal papillomatosis that develops in the skin of many patients with chronic lymphoedema.

Patients with megalymphatics may have chylous ascites, chyluria, chylometrorrhoea, chylothorax or other manifestations of lymphatic fistulae.

SUMMARY

Primary lymphoedema and other syndromes associated with lymphoedema cause significant morbidity. Molecular techniques have greatly improved our understanding of lymphatic specification, lymphangiogenesis, and lymphatic maturation. The relevance of genetic abnormalities in the development of different types of primary lymphoedema is now being elucidated. Increased understanding of these mechanisms will increase the number of candidate genes for genetic testing in both idiopathic inherited and sporadic forms of lymphoedema. Understanding of each of these processes will eventually lead to more effective treatments for disorders of the lymphatic system.

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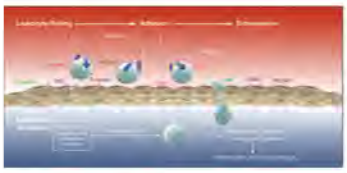

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