Lymphotrophic nanoparticle-enhanced magnetic resonance imaging for nodal clinical target volume delineation in the radiotherapy treatment planning of pelvic malignancies:

Derivation of a class solution nodal clinical target volume

By

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A thesis submitted in conformity with the requirements for the degree of M. Sc.

Graduate Department of the Institute of Medical Science

University of Toronto

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Master of Science  2010

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Dextran-coated ultra-small, superparamagnetic, iron oxide particles (USPIO) have been proposed as magnetic resonance (MR) lymph node contrast agents. This thesis analyzed the topographic distributions of the pelvic and inguinal lymph nodes and quantified their spatial relations with the adjacent vascular system. We hypothesized that USPIO would facilitate identification of normal lymph nodes in a manner superior to that afforded by computed tomography or unenhanced MR, but using current clinically available scanners would be unlikely to identify microscopic nodal metastases. We have constructed a high quality nodal atlas describing probability distributions for lymph node number, size and position. Using this model, we then defined a generic three-dimensional nodal clinical target volume and a means of accurate delineation of this volume in a three-dimensional representation. This is the most quantitative assessment of the pelvic and inguinal lymphatics to date and will help to improve the successful targeting of lymph nodes for radiotherapy.
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<tr>
<td>AP</td>
<td>Anterior-Posterior</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<td>CTV</td>
<td>Clinical Target Volume</td>
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<tr>
<td>DICOM</td>
<td>Digital Imaging and Communications in Medicine</td>
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<tr>
<td>ePLND</td>
<td>extended pelvic lymphadenectomy</td>
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<tr>
<td>FOV</td>
<td>Field of view</td>
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<td>GTV</td>
<td>Gross Target Volume</td>
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<tr>
<td>GU</td>
<td>Genitourinary</td>
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<td>GYN</td>
<td>Gynecologic</td>
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<td>IM</td>
<td>Internal Margin</td>
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<td>IMRT</td>
<td>Intensity modulated radiotherapy</td>
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<td>nCTV</td>
<td>nodal Clinical Target Volume</td>
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<tr>
<td>nodal CTV</td>
<td>nodal Clinical Target Volume</td>
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<td>nPTV</td>
<td>nodal Planning Target Volume</td>
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<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>PA</td>
<td>Posterior-Anterior</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>POP</td>
<td>Parallel Opposed Pair</td>
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<td>PTV</td>
<td>Planning Target Volume</td>
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<tr>
<td>RTOG</td>
<td>Radiation Therapy Oncology Group</td>
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<td>S3</td>
<td>Third Sacral Vertebra</td>
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<tr>
<td>SAD</td>
<td>Short-axis diameter</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SM</td>
<td>Set-up Margin</td>
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<td>Tesla</td>
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<td>T2-w</td>
<td>T2-weighted</td>
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<td>$^{232}$Th</td>
<td>Thorium</td>
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<tr>
<td>US</td>
<td>Ultrasound</td>
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<tr>
<td>USPIO</td>
<td>Ultra-small Particles of Iron Oxide</td>
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<td>VHM</td>
<td>Visible Human Male</td>
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Chapter 1. General Background and Literature Review

Science is the belief in the ignorance of experts.
- Richard Feynman

1.1 Lymphatic Anatomy and Function

The lymphatic system functions within the immunologic system, playing a key role in host defense, in addition to the absorption and transportation of nutrients from the gastrointestinal system and the regulation of interstitial fluid volume. The lymphatic network arises embryologically from the developing blood vessels and functions in parallel with the cardiovascular system\(^1\). The arterial system acts as a conduit to distribute oxygen and other nutrients throughout the body to support tissue growth, development and maintenance. The venous system returns the waste-laden blood to the kidneys, liver and lungs where the products of metabolism are processed and/or eliminated. The fluid that remains within the interstitial space enters the lymphatic system and passes through a network of lymphatic vessels and nodes to ultimately empty into the venous system before reaching the heart.

The lymphatic system architecture is similar to the venous system, with a fine capillary network permeating most tissues and transitioning to successively larger lymphatic vessels\(^1\). Lymphatic vessels possess three layered walls comprised of intima, media and adventitia, as do the veins. Unlike the veins however, the lymphatic vessels tend to
be of much smaller diameter and are difficult to appreciate without the use of specialized imaging techniques\textsuperscript{2, 3}.

Lymph nodes of varying sizes are distributed along the routes of lymphatic return. They are glandular structures, which vary markedly in both size and shape and are comprised of a peripheral cortex and a medullary core (Figure 1.1). The parenchyma is surrounded by a collagenous fiber capsule immediately beneath which is the subcapsular sinus. Trabeculae extend from the subcapsular sinus to the interior and a coarse network of reticular fibers that create an interdigitating sinus system. As with the lymphatic vessels, standard clinical radiographic imaging techniques lack sufficient resolution to detect normal, non-diseased lymph nodes and are unable to discern their inner structure.

Communicating with the intercellular space, in which interstitial fluid resides, the meshed capillary networks afford entry into the lymphatic system\textsuperscript{2, 4}. Lymph arises from interstitial fluid and enters into the blind-ended sacs at the terminus of a given capillary network. Once in the lymphatic network, the lymphatic fluid progresses through lymphatic collectors to reach the lymphatic vessels. The initial lymphatic capillaries, collectors, vessels and nodes that communicate and direct lymph within a particular anatomic region together constitute a lymphatic drainage basin. Adjacent lymphatic basins are often interconnected in complex ways.

Fluid enters the lymphatic network through the route that presents the least amount of resistance. Under normal physiologic conditions, the fluid within a territory flows to the
lymphatics in closest proximity. The system however is complex and lymphatic flow in one region often influences flow in adjacent regions (Figures 1.2, 1.3 and 1.4)\textsuperscript{5, 6}. For example, efferent lymphatic vessels, which act as conduits for lymph exiting a lymph node within a given lymphatic basin, may function as afferent vessels to a node within a different lymphatic basin. These interconnections produce a structural physiologic reserve that is important during times of increased interstitial fluid load.

Lymph nodes are active in the immunologic filtration of lymph and the generation and storage of lymphocytes. During embryogenesis, the volume of nodal tissue involved in the lymphatic drainage for a given region is formed. With growth and development, this volume may be divided into several small volume lymph nodes or a small number of larger volume lymph nodes all of which occupy the fatty tissue around the blood vessels\textsuperscript{7}. As individuals age, the medullary cortex within a lymph node may involute resulting in senile atrophy. While the nodal capsule remains, the node itself may become difficult to detect clinically\textsuperscript{5}. When an immune response is initiated, these nodes may return to function with their peers in a lymphatic basin. Irrespective of the presence or degree of atrophy, lymph will still continue to pass through all of the lymph nodes within a given lymphatic basin.

The histologic architecture of the lymph node interior facilitates its ability to filter lymph fluid. In a given lymphatic territory, lymph will enter into the node through an afferent vessel and empty into the subcapsular sinus. Once in the lymphatic interior, the lymph travels through only a part of the nodal interior with the trabeculae, cortical and medullary sinuses effecting lymph flow direction (Figures 1.1 and 1.4)\textsuperscript{6}. Metastases
entering the subcapsular sinus through an afferent vessel will not be dispersed throughout the entire marginal sinus but instead enter into the porous intermedullary sinuses in proximity to their point of entry into the lymph node. Nodal filtration during the period following metastatic seeding is not markedly impaired as only one region is initially affected. With on-going growth and proliferation, cancer cells may extend throughout the nodal interior and restrict fluid entry by destroying or effacing the lymph sinuses.
Figure 1.1: Diagrammatic representation of a lymph node in cross-section showing the entry and passage of lymph, its blood supply and the distribution of macrophages within its interior.
Figure 1.2: Representation of lymph (blue and yellow) draining from a lymph territory (blue) into a first echelon lymph node shown in cross-section.
Figure 1.3: Schematic depiction of the interconnections between lymphatic capillaries, collectors, vessels and nodes for adjacent lymphatic territories. (Modified from ⁵, ⁶)
Figure 1.4: Schematic of the routes of lymph drainage from the lymphatic territories and the proportion of the territory specific lymph in the lymph nodes downstream. (Modified from ⁶).
Lymph entering peripherally into the lymphatic capillaries will eventually reach a central location of venous return through either the thoracic duct, which empties into the left brachiocephalic vein, or the right lymphatic duct, which empties into the right subclavian vein. Additional lymphaticovenous or lymphovenous anastomoses have been described\textsuperscript{8-18} but are infrequent and tend not to function under normal physiologic conditions. The purpose, frequency and distribution of these anastomoses remains unclear.

The form of the lymphatic system is such that anatomic and/or physiologic disturbances may transpire without markedly compromising its function. Were an obstruction to occur distally, the lymph within the intercellular space would continue to drain to that site which presented the least amount of resistance. In the region of the obstruction, the fluid instead of entering into the obstructed capillary network adjacent to it travels in the direction of lowest pressure and enters into the lymphatic system at some distance from its original location. Similarly, obstruction of lymphatic collectors and afferent and efferent lymphatic vessels tend to alter the route and passage of lymph to what would typically be secondary or tertiary routes of drainage under normal conditions. Intralymphatic growth of tumor metastases will compromise the sinus system within the node and retard lymph entry into it. With an increase in intranodal pressure, lymph will be diverted elsewhere within the system to be filtered by other nodes. An increase in pressure within the lymph vasculature may open previously dormant lymphaticovenous anastomoses. All of these responses are adaptive and facilitate continued functioning of the lymphatic system in spite of local or regional insults or stressors\textsuperscript{4}. 


An appreciation of the form and function of the lymphatic system in its normal and aberrant states is instructive in the pathologic and radiographic assessment of lymph node metastases. Knowledge of the lymphatic drainage of a given organ or region and an understanding of lymphatic physiology affords an insight as to the probable sites of initial nodal spread from cancers arising from within it. Therefore, cancer staging and therapy is predicated upon a sound study and understanding of the lymphatic system.

Despite the importance of the lymphatic system in maintaining normal physiologic function and its role in host defense, research into the lymphatic system lags behind its circulatory counterpart. The single most comprehensive study of the lymphatic system, *Vasorum lymphaticorum corporis humani historia et ichnographia*, was undertaken by Paolo Mascagni. This exhaustive cadaveric study used cannulation and mercury injection to identify the lymphatic vessels (Figures 1.5 and 1.6). Despite the passage of 223 years since its publication, there exists no modern non-invasive or invasive imaging equivalent. While further studies such as those of Rouviere, have helped to describe and refine the patterns and routes of lymphatic transport through the parietal and visceral lymph nodes, Mascagni is credited with identifying and naming approximately 50% of the known lymphatic vessels.
Figure 1.5: (Left) Engraved front plate of *Vasorum lymphaticorum corporis humani historia et ichnographia* by Mascagni, and (Right) apparatus used to infuse mercury into the cadaveric specimens and depictions of the efferent and afferent lymphatics with associated lymph nodes.
Figure 1.6: Representative engraved plates from *Vasorum lymphaticorum corporis humani historia et ichnographia* by Mascagni depicting (Left) the inguinal, iliac and aorto-caval lymphatic vessels and nodes within the pelvis and retroperitoneum and (Right) the left inguinal region showing the exquisite detail inherent in this work.
While no comparable study has been done in the modern era, the National Institute of Health has recorded post-mortem anatomy using digitized photomacrographs of male and female cadavers subjected to serial axial macrotomal sectioning\textsuperscript{20}. The Visible Human Male data set that resulted from this process is comprised of axial images with pixel dimensions of 0.14 mm $\times$ 0.14 mm and 1 mm axial slice spacing, and the Female data set with pixels measuring 0.33 mm $\times$ 0.33 mm and 0.33 mm axial slice spacing. The inherent natural contrast between adjacent anatomic structures facilitates both manual and automatic segmentation\textsuperscript{21}. Delineation of the structures of interest and post-processing can provide 3-dimensional rendering of the lymph nodes, blood vessels, organs and clinical volumes of interest (Figure 1.7). While these are high fidelity virtual representations of lymph nodes, organs and other anatomic structures of interest, the lymphatic system vasculature is too fine and lacks sufficient inherent contrast to be readily discernable even at this resolution.

In contemporary clinical practice, there are no methods for the \textit{in vivo} detection of the lymphatics (lymphatic capillaries through to lymph nodes and efferent lymphatic vessels) of the inguinal and pelvic region in their entirety. This has profound implications for the investigation and management of individuals with disorders and diseases involving the lymphatic system. In the absence of a comprehensive image based means for patient specific assessment, incorporation of information that can be obtained from nuclear medicine and diagnostic radiology studies with knowledge of lymphatic structure and function is the norm.
Figure 1.7: (a) High resolution pelvic axial section from the Visible Human Anatomic Series illustrating (b) small lymph nodes (arrows) close to the external iliac vessels. (c) Three-dimensional computer rendering derived from this dataset showing the relationship between 177 pelvic and para-aortic lymph nodes (green) and the adjacent vessels (red and blue).
1.2 Pelvic Lymphatic Network

Pelvic tumors are among the most common malignancies in women and men and are associated with a significant burden of suffering worldwide\textsuperscript{22-24}. Many pelvic tumors including prostate, urinary bladder, uterine, cervical, vulva and anal canal cancers frequently spread via the lymphatic channels to pelvic and inguinal lymph nodes. Lymph node involvement at diagnosis generally is associated with a high risk of metastatic disease at other sites outside of the pelvis and lower overall survival rates following treatment. In patients undergoing surgical treatment, pelvic lymph node dissection is frequently performed at the same time as removal of the primary tumor. Lymph node dissection may be therapeutic as well as providing valuable information about prognosis and the need for additional treatment like chemotherapy\textsuperscript{25-48}. In patients receiving radical or adjuvant radiotherapy, pelvic and inguinal lymph nodes are often included in the clinical target volume (CTV) with the aim of reducing recurrence and improving survival. The success of radiotherapy in this context is dependent on a detailed understanding of pelvic and inguinal lymph node anatomy and patterns of cancer spread.

The pelvic lymphatics are divisible into two primary networks of lymph nodes and connecting lymph vessels: parietal and visceral\textsuperscript{25, 26, 28-30, 49-51}. The parietal network of lymphatics receives lymph from the walls of the pelvis that drain the perineum and muscles of the pelvic girdle. The visceral network drains the pelvic organs via a series of vessels and nodes that are situated in close relation to the pelvic arteries and veins. The visceral lymphatics can be divided into major groups based on their anatomic
relation to the pelvic vasculature: common iliac, internal iliac, external iliac, superficial inguinal and deep inguinal (Figures 1.8, 1.9, 1.10 and 1.11). The following subsections describe in more detail the lymphatic architecture of the regions of relevance to this body of work.

1.2.1 Common Iliac Lymphatics

The common iliac lymph nodes (4 to 7 nodes in most people) are in close proximity to the common iliac vessels and comprise three distinct groups: the medial, intermediate and lateral common iliac lymph node chains\textsuperscript{49, 51}. The medial chain occupies the region on the inner aspect of the common iliac artery and forms a triangular arrangement with the corresponding nodes of the opposite side. The intermediate chain (3 to 4 nodes) is located on the posterior aspect of the common iliac artery and vein\textsuperscript{7}. The lateral chain (2 nodes) is positioned along the lateral aspect of the common iliac artery and the medial border of the psoas muscle\textsuperscript{4, 7, 52}. The lateral and intermediate common iliac chains receive drainage from the lateral and intermediate external iliac lymph node chains and empty into the lateral aortic lymph node chains\textsuperscript{52}. The medial common iliac lymph node chain may receive lymphatic drainage directly from the bladder neck and cervix uteri\textsuperscript{7}. 
Figure 1.8: Frontal projection of the distal lumbar spine and bony pelvis depicting the major vascular segments of interest.
Figure 1.9: Frontal oblique projection of the distal lumbar spine and bony pelvis depicting the major vascular segments of interest.
1.2.2 Internal Iliac Lymphatics

The internal iliac nodes are associated with the internal iliac vessels and their branches. They are situated anterior to the sacro-iliac joint descending to the level of the greater sciatic foramen. The most superior node, the superior gluteal, is located in proximity to the origin of the superior gluteal artery. The inferior most node of this chain is situated between the umbilical and obturator arteries. Intermediate nodes may be located along the uterine, internal pudendal, inferior gluteal and middle rectal arteries. The internal iliac lymph nodes drain the pelvic viscera including the posterior aspect of the prostate, inferior and lateral aspects of the bladder, seminal vesicles, middle and inferior aspects of the vagina and the body of the uterus. Lymphatic drainage from the perineum, inferior prostate and lower vagina travels to the internal iliac chain. The efferent vessels from the internal iliac nodes ascend superiorly through the hypogastric lamina and pass below the common iliac vein to the intermediate group of common iliac nodes.

1.2.3 External Iliac Lymphatics

The external iliac nodes are continuous with the external iliac vessels forming three separate chains: lateral, intermediate and medial. The lateral external iliac chain is situated within the cleft formed by the medial border of the psoas muscle and the lateral aspect of the external iliac artery. The inferior most node of this chain (lateral lacunar node) is situated deep to the inguinal ligament. The intermediate external iliac chain is situated on the medial side of the external iliac artery and anterior to the external iliac
vein. The medial external iliac chain is situated on the medial and dorsal aspect of the external iliac vein abutting the lateral pelvic sidewall. The external iliac lymphatics primarily drain the lower limbs receiving lymphatic fluid from the superficial and deep inguinal nodes\textsuperscript{4, 7, 53}. Of note, the medial and intermediate chains also receive drainage from the obturator lymphatics, which in turn receive the visceral lymphatic vessels arising from the lateral lobes of the prostate gland, bladder fundus, cervix uteri and upper part of the vagina\textsuperscript{53}. The juxtavisceral nodes of the vagina and cervix primarily form associations with the medial group of external iliac nodes\textsuperscript{54}.

1.2.4 Superficial Inguinal Lymphatics

The superficial inguinal nodes, anterior to Scarpa’s femoral triangle, typically consist of 10 to 12 large nodes\textsuperscript{7, 49, 54}. They are divided from the femoral nerve, femoral vessels and the deep inguinal nodes by the cribiform fascia. The medial superficial inguinal nodes receive afferent vessels from the vulva and inferior vagina\textsuperscript{54}. Additional drainage from the uterine horns, which travels through the inguinal canal to reach these nodes, may also occur\textsuperscript{54}.

1.2.5 Deep Inguinal Lymphatics

The deep inguinal lymph nodes are located medial to the femoral vein and posterior to the fascia lata in the femoral canal\textsuperscript{49, 51}. The superior most deep inguinal node may reside in the low pelvis and has been referred to as Cloquet’s or Rosenmuller’s node\textsuperscript{7}. 
The drainage from the deep inguinal nodes is through the femoral canal to the lacunar nodes of the external iliac chain.

### 1.3 Lymphatic Drainage of the Pelvic Organs

The lymphatic drainage of the prostate gland, urinary bladder, corpus uterus, cervix, vulva and anal canal has been studied using a variety of approaches, including detailed anatomic dissection and surgical staging of patients with cancer to define the distribution of nodal metastases. Typical patterns of pelvic lymphatic flow are illustrated in Fig. 1.12. However, lymphatic drainage can be highly variable from patient to patient, influenced by differences in the distribution of normal lymphatic vessels and the emergence of collaterals following destruction or obstruction of the usual routes of flow. This can occur, for example, following surgery that disrupts lymphatic vessels or as a result of bulky cancer lymph node metastases. Nevertheless, commonalities emerged from the anatomic and surgical studies that influenced conventional radiation treatment planning for many years prior to the widespread implementation of highly conformal techniques like intensity-modulated radiotherapy (IMRT).

The volumes irradiated with conventional radiotherapy field borders are typically much greater than those defined for highly conformal treatments. While this limits the dose that may be delivered and sparing of adjacent normal tissues, it does afford greater assurance of target coverage. In transitioning to increasingly conformal therapies, an
Figure 1.10: Frontal projection of the distal lumbar spine and bony pelvis depicting the major vascular segments of interest within the femoral triangle.
Figure 1.11: Diagrammatic representation of the distribution of the deep and superficial inguinal lymph nodes within the femoral triangle.
understanding of lymphatic topography and routes of lymph flow is of critical importance. Disease site-specific and patient-specific knowledge of lymphatic drainage is becoming increasingly important to assure adequate radiotherapy targeting of critical lymph node regions.

1.3.1 Lymphatic Drainage of the Prostate Gland

Lymphatic drainage originates within the glandular acini of the prostate, traverses the prostatic capsule and forms lymphatic networks on the posterior and superior aspects of the gland\(^7\), \(^{49, 53, 55, 59-64}\). Lymphatic flow from the anterior lobe of the prostate occurs primarily via the medial and intermediate external iliac chains through direct lymphatic vessels or after passing through the prevesical nodes\(^7, 49, 53, 55\). Drainage posterior along the course of the internal pudendal artery to the inferior gluteal nodes may also occur\(^7, 49, 53, 55\). The posterior lobe of the prostate is drained by three lymphatic trunks\(^53\). The first arises from the base of the gland, follows the seminal vesicles, passes superior to the terminal segment of the ureter and drains into the middle and superior nodes of the intermediate external iliac chain. The second lymphatic pathway originates from the posterior and inferior regions of the prostate gland and follows the prostatic artery to empty into the internal iliac lymph nodes. The third travels posterior to the superior gluteal, lateral sacral and promontory lymph nodes along the course of the sacro-prostatic ligament\(^60\). The lymphatic drainage of the prostate is known to have many anastomoses, which may result in flow through the lymphatics of the rectum, bladder, ampulla of the ductus deferens and seminal vesicles\(^53\). Lymph from the seminal vesicles combines with the efferent
Figure 1.12: Diagrammatic representation of the distribution and routes of lymphatic drainage for the inguinal and pelvic lymph nodes. (Modified from ⁴).
lymphatic vessels of the urinary bladder and prostate to drain into the intermediate and medial external iliac nodes as well as the internal iliac nodes.

1.3.2 Lymphatic Drainage of the Urinary Bladder

An elaborate network of lymphatic drainage is present within the mucosa and muscular layers of the bladder. The mucosal lymphatics unite to form collecting lymph vessels that drain initially to the prevesical space and paravesical lymph nodes before reaching the middle and superior nodes of the medial and intermediate external iliac groups\textsuperscript{7, 53, 63, 65-67}. Clearance of lymph from the anterior aspect of the bladder wall begins with collecting lymph vessels situated in proximity to the middle third of the lateral vesicle border. Once the lymph fluid has reached this location, it flows through vessels towards the origin of the umbilical artery in addition to the superior vesical artery. The lymph vessels from the posterior bladder wall unite with vessels from the lateral vesicle border to reach the external iliac nodes. Additional lymph vessels arise from the posterior bladder wall and course either to the posterolateral angle of the bladder and then to the medial and intermediate external nodes or extend to the lymph vessels of the bladder trigone. Less commonly, lymph may flow to the medial lacunar node or internal iliac nodes. The bladder trigone predominately drains through vessels located along the course of the uterine artery to the medial and intermediate groups of external iliac nodes\textsuperscript{53, 68}.
1.3.3 Lymphatic Drainage of the Corpus Uterus

Lymphatic networks exist within the mucosa of the corpus uterus in addition to the muscularis and subserosa\textsuperscript{53, 54}. Lymph vessels emerge from the lateral aspects of the uterus to travel along the suspensory ligaments in association with the ovarian branches of the uterine artery. Ultimately, they unite with the tubo-ovarian lymph vessels and terminate in the lumbar nodes close to the renal and superior mesenteric arteries at the level of the lower poles of the kidneys\textsuperscript{4, 7, 69-72}. Lymphatic drainage may also occur laterally to the external iliac lymph nodes, with associated drainage to parauterine nodes and via lymphatic trunks in the broad ligament to the superior interiliac lymph nodes. Drainage channels arising in proximity to the cornua, mesosalpinx and tubular bed direct lymph to the aortic and superior gluteal nodes. Lymph vessels at the site of insertion of the round ligament may drain to the superomedial group of superficial inguinal nodes. Anastomoses among lymph vessels arising in the uterus may also result in drainage into the external iliac and superior gluteal lymph nodes\textsuperscript{4, 54}.

1.3.4 Lymphatic Drainage of the Cervix

A rich lymphatic network exists within the uterine cervix and the adjacent parametrial tissues, which terminates in three principal collecting lymphatic vessels\textsuperscript{54, 63, 69, 71, 73-76}. They include: the preureteral collecting vessels found anteriorly; the retroureteral vessels; and the uterosacral vessels posteriorly\textsuperscript{7, 54, 73}. The anterior preureteral lymph vessels course in close association to the uterine artery and travel anterior to the ureters. They comprise the primary lymphatic chain of the cervix and terminate in the
superior and middle nodes of the medial and intermediate external iliac chains. Less commonly, they may also drain into the obturator group of external iliac lymph nodes. Traveling with the uterine vein, the retroureteral lymph vessels pass posterior to the ureter and end in the internal iliac lymph nodal chain. Drainage to the promontory nodes may also occur. The uterosacral collecting lymph vessels travel posterolateral to the rectum and then superiorly to reach the lateral sacral, promontory and subaortic lymph nodes. Uninterrupted drainage from the cervix to the aortic nodes has been observed via vessels traveling in association with the ureter to the common iliac, subaortic and aortic nodes at the level of the pelvic brim. The lymphatic drainage of the uterine cervix may be direct or may be interrupted by passage through the parauterine and paravaginal lymph nodes. The ureterouterine node, located at the junction of the ureter and the uterine artery, is the largest of the parauterine and paravaginal lymph nodes. Intramural and parauterine anastomoses of the cervical lymphatics occur with the lymphatic vessels of the corpus uterus and vagina.

1.3.5 Lymphatic Drainage of the Vulva and Anal Canal

Lymphatic drainage from the vulva typically proceeds to the medial superficial inguinal lymphatics before reaching the deep inguinal lymph nodes and then the external iliac lymph nodes. Vulvar lesions in the mid-line, in proximity to the clitoris, may drain to either or both sides and can drain directly into the external iliac lymph nodes effectively bypassing the inguinal ones. Similarly, posterior lesions may drain directly into the sacral lymph nodes. Lymph from the anal canal may drain into either the
superficial inguinal lymphatics or through the lower rectal lymphatics and the internal iliac and inferior mesenteric lymph nodes\textsuperscript{7, 53, 55}.

1.4 Imaging of Lymphatics

The variable nature of the lymphatic drainage from the prostate gland, urinary bladder, corpus uterus, cervix, vulva and anal canal implies the need for patient-specific lymphatic imaging to define individualized target volumes for highly conformal radiation treatment. Unfortunately, the fine reticular structure of the small lymph vessels and nodes, which comprise a significant portion of the lymph vascular system, limit the usefulness of currently available conventional imaging approaches.

Early radiographic visualization of the lymphatic system followed 35 years after Roentgen’s discovery of x-rays. In 1930 and 1931, Funaoka and Carvalho each injected thorium (\textsuperscript{232}Th) dioxide into the subcutaneous tissues and lymph nodes to demonstrate them radiographically\textsuperscript{77, 78}. While images were obtained from this technique, the unstable \textsuperscript{232}Th nuclide decays by alpha particle emission making the imaging agent highly carcinogenic. It was not until 1952, when Kinmonth used a water-soluble contrast media that was injected into cannulated lymphatic vessels to study lymphedema, that a practicable means of imaging became available\textsuperscript{52}. Injection of contrast material was preceded by subcutaneous injection of a vital dye to opacify the small lymphatic channels at the local site and facilitate their cannulation\textsuperscript{52}. While the lymphatic vessels could be demonstrated, the water-soluble contrast agents were limited in their ability to visualize the lymph nodes because of rapid dilution and clearance. Malek in 1959
revised the technique developed by Kinmonth and employed an oil-soluble iodized contrast material that remained stable in tissue for longer periods\textsuperscript{79}. Prolonged retention within the lymphatic system enabled oil-based media to delineate a far greater extent of the lymphovascular system along the drainage path from the site of injection. Cannulation of the lymphatics in the distal lower extremity allowed depiction of lymphatic channels and nodes in the pelvis and abdomen. In addition, lymphangiography allowed visualization of the internal structure of lymph nodes, and the detection of small metastases that appeared as filling defects on an otherwise uniform background of contrast uptake. Lymphangiography was a major step forward compared to earlier imaging techniques, and was used clinically for many years to delineate lymph nodes in patients with cancer and other ailments.

While lymphangiography had many advantages, it also had limitations that largely were imposed by the anatomic structure and architecture of the lymphovascular system\textsuperscript{58}. Cannulation of the lymphatic vessels was invasive, the injection of the agent was uncomfortable and needed to be done over an extended period and the distribution of the agent in tissue was determined by patterns of lymphatic drainage from the site of injection. Clinically relevant lymph nodes anatomically and functionally remote from the primary drainage pathway often were not visualized. For example, the internal iliac lymph nodes, to which central pelvic tumors frequently metastasize, were difficult to discern following injection of lymphatic channels in the leg that drain mainly via the inguinal and external and common iliac pathways\textsuperscript{2,49,52}. 

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With the emergence of cross-sectional imaging, the routine use of direct lymphangiography waned. Technologic improvements in ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) supplanted the routine use of lymphangiography in the provision of clinical care and in research. Ultrasound can detect imaging features indicative of macrometastases in superficial lymph nodes, including irregularities in external contour, enlargement, round shape and loss of the central hilum\(^\text{80}\). Doppler flow can also provide useful ancillary information. Ultrasound imaging does however lack a means of providing easily interpretable serial image data sets. CT and MRI, while non-invasive cross sectional imaging modalities, do not have sufficient spatial and contrast resolution to detect all of the lymphatic channels and normal lymph nodes. Neither technique is able to identify nodal metastases less than a few mm in size\(^\text{81-86}\). Evolving dynamic CT and MR imaging approaches may improve the detection of small volume lymph node metastases\(^\text{87-89}\). While these modalities have unique strengths in lymphatic imaging, they fail to capture completely the information that what was previously available from lymphangiography.

Molecular imaging approaches provide the promise of a new paradigm. The non-invasive measurement of patient specific tumor physiology has the potential to improve staging and response assessment following radiotherapy and other anti-cancer treatments and facilitate the implementation of new molecular targeted therapeutic strategies. The use of the glucose analogue 18F-fluoro-2-deoxy-d glucose (FDG) with Positron Emission Tomography (PET) has been shown to improve the detection of lymph node metastases in many malignancies\(^\text{90-109}\), reflecting the higher glucose uptake and glycolytic rates in tumors compared to most normal tissues\(^\text{110}\). A disadvantage of
PET is low spatial resolution that can partially be overcome by co-registration with CT images to enhance the localization of lymph node metastases\textsuperscript{109}. However, PET is limited by an inability to consistently detect disease in sub-centimeter lymph nodes and micrometastatic lymphatic spread\textsuperscript{111}.

1.4.1 USPIO-Enhanced MR Lymphatic Imaging

A novel class of MR imaging agents that capitalizes on the properties of iron within a magnetic field has been explored clinically\textsuperscript{112-122}. Dextran-coated ultra-small, superparamagnetic, iron oxide particles (USPIO) have been evaluated as MR lymph node contrast agents for discriminating between malignant and benign lymph nodes. The particles are injected intravenously and taken up by macrophages in the reticuloendothelial system, most of which reside in lymph nodes. The iron particles cause a local change in tissue susceptibility, which results in a signal void or dark region on post-contrast T1-weighted and T2-weighted MR images. Uniform signal loss is apparent in normal lymph nodes as well as in those that are enlarged due to inflammation. However, metastatic tumor in lymph nodes appears as a persistent bright focus on a darker background in post-contrast scans, reflecting reduced regional macrophage infiltration and USPIO uptake in cancer (Figures 1.13 and 1.14).

The potential of USPIO as a negative contrast agent for detecting lymph node metastases has been explored with some success. USPIO-enhanced MRI has been shown to improve the identification of lymph node metastases in patients with pelvic malignancies relative to standard CT and/or MR techniques. However, it has limited
sensitivity for the detection of metastases in lymph nodes <5 mm in size, and is unable to identify microscopic nodal disease with the accuracy of surgical staging\textsuperscript{123}. An important advantage though, in the context of radiotherapy treatment planning, is the capability of USPIO-enhanced MR to facilitate the identification of apparently normal lymph nodes relative to CT or unenhanced MR\textsuperscript{117, 124}. The improved identification of these nodes could aid in the definition of lymph node target volumes for high-precision adjuvant treatment of patients at high risk of harboring occult nodal metastases.
Figure 1.13: (A) diagrammatic representation of a normal lymph node in cross-section following USPIO administration and the corresponding MR appearance of lymph node. (B) lymph node with a moderate cancer burden and its post-contrast MR appearance. (C) lymph node with a minimal cancer burden and its post-contrast MR appearance.
Figure 1.14: (a) T2-w pre-contrast axial MR image of the pelvis at the level of the acetabulum. Inset figure depicts a normal-sized proximal inguinal lymph node with similar signal characteristics to the surrounding tissues. (b) T2-w post-contrast MR image showing uniform distribution of USPIO contrast agent as evidenced by the pattern of signal loss evident in the inset image.
1.5 Radiotherapy of Pelvic Lymph Nodes

Radiotherapy has a proven role in the curative management of gynecological and genitourinary malignancies\textsuperscript{125-134}. It may be used as primary treatment with the intent of eradicating gross disease or in an adjuvant fashion after primary surgery to reduce the likelihood of recurrence in high-risk patients. Tumors of the prostate gland, urinary bladder, corpus uterus, cervix, vulva and anal canal commonly metastasize to iliac or inguinal lymph nodes, and irradiation of these nodes has been shown to enhance pelvic control and/or improve patient survival\textsuperscript{39, 135-150}. Historically, pelvic radiation fields that encompassed lymph nodes were planned using general knowledge of patterns of lymph node spread, derived from anatomic and surgical staging studies as discussed previously. High-resolution imaging of lymph node anatomy that could be applied to individual patients was not available, nor was the technology to allow precise radiation treatment targeting. Therefore, treatment plans tended to be very simple with four-field, three-field or a two-field (AP/PA POP) beam arrangements positioned using bony anatomy. The treatment volumes generally are quite large to minimize the chance of missing disease. However, large volumes of normal tissue were also irradiated, which contributed to side effects and limited the dose of radiation that could be used safely\textsuperscript{151-153}.

Radiotherapy treatment planning is now largely based on CT imaging rather than planar x-rays. CT affords an ability to obtain high contrast as well as high resolution images with a marked improvement in the level of anatomic detail that is appreciable in comparison to projection radiography. With this improvement in imaging, standardized
definitions have been developed to facilitate treatment uniformity and standardization (Figure 1.15). The Gross Target Volume (GTV) constitutes the clinically appreciable tumor extent with the Clinical Target Volume (CTV) being the volume that includes microscopic spread into adjacent tissues. The CTV includes direct spread from the GTV in addition to spread to local and regional lymph nodes (nodal Clinical Target Volume - nCTV). A further geometric margin of expansion (Planning Target Volume - PTV) is then applied to account for physiologic movement (Internal Margin - IM) and interfraction differences in treatment reproducibility (Set-up Margin - SM). Derivation of a treatment plan using these definitions will produce a 3D radiotherapy treatment volume that not only accurately and reproducibly encompasses the key target regions but also defines the regions that can be avoided so as to spare critical anatomical structures.
Figure 1.15 Depiction of radiotherapy target volumes: GTV - Gross Target Volume, CTV - Clinical Target Volume, PTV - Planning Target Volume, IM - Internal Margin, and SM - Set-up Margin.
The use of planning CT has lead to improved targeting of the primary tumor but has not substantially influenced the delineation of nodal volumes because conventional CT and MRI scans are imperfect in their ability to detect pelvic lymph nodes that are not enlarged\textsuperscript{29, 154, 155}. However, the majority of the pelvic lymph nodes lie in close proximity to adjacent blood vessels. Unlike the lymph nodes, the blood vessels are more readily discernable with either CT or MR imaging. Therefore, several investigators have proposed using blood vessels as surrogates for nodal position with the application of an appropriate margin of expansion to derive nodal clinical target volumes (nCTV) for radiotherapy\textsuperscript{156-159}. These margins could either be isotropic (uniform in all radial directions around a vessel cross-section) or anisotropic (non-uniform). The ideal nCTV margin would include all clinically relevant nodal tissue, while excluding as much surrounding normal tissue as possible.

Several studies have now demonstrated that standard pelvic radiotherapy fields based on bony anatomy often fail to encompass all clinically relevant lymph nodes, despite large treatment volumes\textsuperscript{160-163}. Bonin \textit{et al.} used lymphangiography to study the adequacy of pelvic lymph node coverage, as defined by the Gynecologic Oncology Group, in patients with cervical carcinoma\textsuperscript{161}. Application of standard field borders resulted in inadequate coverage of the lower, lateral external iliac lymph node in 45\% of patients. In a similar study, Finlay \textit{et al.} delineated pelvic blood vessels as a surrogate for the lymphatics in 43 patients with cervix cancer\textsuperscript{160}. With standard pelvic fields, there was inadequate lymphatic coverage at the level of the bifurcation of the common iliac arteries in 79.1\% of patients, inadequate lateral coverage of the external iliac arteries in
20.9% of patients, and inadequate anterior coverage of the external iliac arteries in 69.7% of patients\textsuperscript{160}. In addition, 44.2% of patients were judged to have excessive lateral coverage (unnecessarily wide anterior-posterior fields >2 cm lateral to the external iliac artery), 14% had excessive anterior coverage and 9.3% had excessive superior coverage. Similar studies using lymphangiograms and surgical clips have corroborated these findings\textsuperscript{162, 163}. Standard pelvic fields do not adequately treat portions of the common and external iliac lymph nodes and may result in irradiation of larger than necessary volumes of normal tissue. These studies did not directly address the implications of these results on subsequent disease recurrence in the pelvis, although the highly variable nature of lymph node metastatic spread in individual patients implies that all potential sites of disease need to be encompassed within the high dose radiation volume to achieve maximal benefit.

There have been major advances in radiation treatment delivery over the past several years that have revolutionized the ability to deliver dose in a highly conformal manner to specific targets, while at the same time excluding surrounding normal tissues. Figure 1.16 is an example of an intensity modulated radiotherapy (IMRT) plan for the treatment of cancer of the anal canal and the inguinal and pelvic lymph nodes, illustrating steep dose gradients at the edge of the high dose volume leading to improved sparing of the sigmoid and high rectum, small bowel and bladder. It is anticipated, as the long term results following IMRT delivery become available, that reductions in late toxicity will become apparent because of improved normal tissue avoidance. Similarly, the potential for dose escalation afforded by IMRT should facilitate improvements in local control and cure. High precision treatments like this highlight the need for accurate primary and
nodal target volume delineation because of the increased potential to underdose tumor. A comprehensive and reproducible pelvic and inguinal nCTV definition would allow the potential of IMRT to be fully exploited to improve tumor control and reduce toxicity.
Figure 1.16 Conventional radiotherapy plan for a carcinoma of the anal canal radiotherapy (top) and an intensity modulated plan (bottom) illustrating a steep dose gradient at the edge of the high dose volume (green) and reduced dose to bladder (B) and genitalia (G).
1.6 Hypothesis, Rationale and Objectives

Radiation therapy has entered a new era of image guidance and precision treatment delivery, which demands accurate and reproducible primary tumor and nodal target volume delineation for optimal results. The determination of nodal target volumes has been particularly challenging because of the anatomic and structural nature of the lymphatics and the difficulties of imaging with conventional techniques. However, USPIO-enhanced MR imaging now offers the potential of improved lymph node delineation, thereby enabling more comprehensive anatomic studies of the lymphatic system. The inherent limitations of USPIO contrast agents and existing MR technology preclude the identification of microscopic lymph node metastases, which are important therapeutic targets in patients undergoing radiotherapy for many pelvic malignancies. Currently, neither this approach nor any other clinically applicable imaging technique offers sufficient sensitivity to be used as a means of targeting only tumor-bearing lymph nodes. Nevertheless, the capability of USPIO-enhanced MR to facilitate detection of apparently normal lymph nodes (sub-centimeter size with uniformly low signal on post-contrast imaging – Fig. 1.13) that may or may not contain microscopic tumor deposits could be utilized to develop more comprehensive radiotherapy target volume definitions.

Ideally, every patient requiring irradiation of pelvic lymph nodes would first have a USPIO-enhanced MR imaging study to define the lymph node target volume. This currently is not practical, however, because of limited MR availability in many centers and lack of a clinically approved USPIO contrast agent. An alternative is to use USPIO-enhanced MR in a large cohort of representative patients to study pelvic lymph node
distribution in relation to more easily imaged anatomic surrogates like the tumor, pelvic and inguinal vasculature, and derive robust three-dimensional population-based nodal target volume definitions that could be adapted to individual patients. The application of these anatomic models in clinical practice would ensure a high probability of accurately targeting volumes at high risk of harboring disease, thus avoiding a geographic treatment miss. In parallel, it would facilitate the avoidance of adjacent normal structures and limit treatment toxicity in the context of a highly conformal radiotherapy plan.

The fundamental hypothesis of this thesis is that USPIO-enhanced MR imaging will facilitate the derivation of a clinically relevant, population-based pelvic and inguinal lymph node target volume definition for radiotherapy treatment planning based on anatomic surrogates for lymph node location. The thesis will focus on nCTV definitions relevant to the treatment of prostate, bladder, uterine, cervical, vulvar and anal cancers. These tumors drain mainly to the inguinal and/or iliac lymph nodes. Pelvic tumors that have a significant component of drainage to mesenteric lymph nodes, such as rectal cancer, will be excluded because of the greater potential mobility of these nodes and concerns about the applicability of this methodology.
The specific aims of this study are to:

1. Identify iliac and inguinal lymph nodes using USPIO-enhanced MRI

2. Define the topographic relationships and spatial probability distribution functions for the location of lymph nodes relative to the adjacent vasculature and other anatomic surrogates.

3. Derive population-based nCTV’s for the treatment of pelvic and inguinal lymph nodes that can be adapted to individual patients based on the location of anatomic lymph node surrogates.

2.1 Introduction

Lymph node metastases are a common site of spread in pelvic malignancies. Patients with a pelvic primary and lymph node metastases at diagnosis have a significantly worse prognosis as compared to those with no evidence of lymphatic involvement. Metastases to local and distant lymph nodes play a major role in tumor staging, therapy and prognosis. Improvements in image-guided, intensity-modulated radiotherapy (IMRT) treatment planning and delivery may help to overcome the adverse prognostic significance of loco-regional lymph node metastases, and contribute to improved patient outcome.

A barrier to the widespread implementation of IMRT for pelvic malignancies is the absence of an objective description of lymph node locations in three-dimensional space that is easily used for radiation treatment planning. Conventional CT and MR do not reliably detect small pelvic lymph nodes, whether normal or abnormal, and have a low sensitivity for identifying metastases, particularly in nodes <10 mm in short axis dimension. Anatomic and embryologic studies have demonstrated the close developmental and spatial relationships between the lymphatics and vasculature. The
pelvic vessels are readily detected with contrast-enhanced CT or MR, and have been advocated as surrogates for lymph node location during treatment planning\textsuperscript{168-170}. However, detailed information about the spatial distribution of nodal tissue in relation to the vessels at different anatomic levels in the pelvis is lacking.

Dextran-coated ultra-small, superparamagnetic, iron oxide particles (USPIO) have been evaluated as MR lymph node contrast agents for discriminating between malignant and benign lymph nodes\textsuperscript{171-173}. While USPIO has the potential to improve the identification of lymph node metastases in patients with pelvic malignancies relative to standard CT and/or MR techniques, it is unlikely that USPIO will be able to identify microscopic nodal metastases with the accuracy of surgical staging\textsuperscript{124}. USPIO has however been shown to facilitate the identification of normal pelvic lymph nodes relative to CT or unenhanced MR\textsuperscript{117}. The improved identification of these nodes using USPIO and MR offers the potential of defining patient-specific nodal CTV’s in a minimally invasive manner that accounts for the risk of microscopic disease, which cannot be imaged.

The aims of this study were to: 1) construct a high quality nodal atlas describing probability distributions for normal and diseased pelvic lymph node number, size and position based upon a 55 patient sample, and 2) define the potential 3D nodal CTV’s to be irradiated and a means of accurate delineation of these volumes in a three-dimensional representation.
2.2 Materials and Methods

This was a single center pilot study eligible to patients with histologically confirmed endometrial, cervix, prostate or bladder cancer with no distant metastases. To ensure uniformity of the sample and to control for artifacts and lymphadenopathy, all subjects were screened to exclude: men with histologically confirmed carcinoma of the prostate and a PSA $\leq 4.0$ or $>100$; evidence of distant metastasis (M1); prior radical surgery or cryosurgery for carcinoma of the prostate; previous radiation therapy; previous hormonal manipulation or chemotherapy; patients with biopsy-proven lymph node involvement; women with histologically confirmed carcinoma of the endometrium or cervix who have undergone recent pelvic surgery with the exclusion of colposcopy, dilatation and curettage or biopsy; men or women with histologically confirmed carcinoma of the bladder who have undergone recent pelvic surgery with the exclusion of TURBT; previous or concurrent cancers other than superficial basal or squamous cell skin carcinoma unless disease free for at least 5 years; or a hip prosthesis.

2.2.1 Patient characteristics

The 55 patients included in this prospective study had a biopsy proven pelvic malignancy. The median patient age was 62 (range 48-80) years. There were 12 women and 43 men (36 prostate cancers, 9 bladder cancers, 5 endometrial cancers, and 5 cervical cancers). None had previously undergone pelvic lymph node dissection or received radiotherapy or chemotherapy. The study was approved by the Research
Ethics Board of the University Health Network and written informed consent obtained. The results of the study were not used in therapeutic decision making for these patients.

2.2.2 MR imaging

MR was performed using a 1.5 T magnet (GE EXCITE 1.5 T, Waukesha, WI, USA) with a four-channel pelvic phased-array coil. Patients were instructed to take nothing by mouth four hours prior to the study. No other preparation was undertaken. Axial imaging sequences were obtained before and 24-36 hours after contrast administration at 3 mm intervals through the pelvis. The pulse sequences consisted of T2w FSE (TR/TE 6500/80; field of view 24-28 cm), and T2*w dual echo GRE (TR/TE1/TE2 1400/14/21 ms; field of view 26-28 cm). Pixel and voxel sizes ranged from 0.9 to 1.2 mm² and 2.7 to 3.6 mm³ respectively. Motion artifacts from peristalsis were minimized with the administration of hyoscine-N-butylbromide (Buscopan) 30 mg or glucagon 1 mg i.v. or i.m. prior to each of the MR imaging sessions. MR imaging remained uniform with no changes in equipment or technique over the duration of the study period.

2.2.3 Ferumoxtran-10

Ferumoxtran-10 was reconstituted from a lyophilized powder. A dose of 2.6 mg Fe/kg was dissolved in 100 ml of normal saline (0.9% NaCl) and infused intravenously.
2.2.4 Image analysis

Analysis of the DICOM images before and after contrast infusion was performed using 3D-DOCTOR® (Able Software Corp., Lexington, MA). The lymph nodes, pelvic vasculature, as well as musculoskeletal landmarks for the sub-aortic/presacral space and the obturator fossa/lateral pelvic sidewall were manually delineated by a single observer (R.D.) and reviewed by a radiologist. Lymph node size evaluation was undertaken using the T2w FSE image series as opposed to the T2*w dual echo GRE which is subject to USPIO induced susceptibility artifacts. To verify the identification and anatomic accuracy of the manual operator (R.D.) defined regions of interest (vascular segments and lymph nodes) an expert abdominal-pelvic radiologist (M.H.) reviewed 10 (18%) of the image datasets. No noted variation between the observers was apparent with regards to either nodal identification and delineation or vessel segment identification. Voxels defining the edge of each distinct vessel and node were identified in the x- and y- planes for each axial slice (z-plane). The x-y-z coordinates defining the segmented structures were then used to generate a 3D stacked image model.

2.2.5 Vascular segmentation

All image sets were reviewed with partitioning of the image data to define the vascular and lymphatic structures of interest. Manual tracing of the vessel walls was undertaken using continuous sampling with vertices recorded along the path length. A closed spline curve was then generated by connection of the vertices. Object specific boundaries on each axial slice were integrated through the series to define a three-dimensional
volume. All points (x, y, and z) at the boundary edge and those pixels enclosed by these points were considered to be part of the object. Those points and pixels not part of a target structure were considered to be background. Window width and window level were standardized to ensure optimization of contrast and brightness.

Segmentation of the pelvic arterial and venous system was performed in a caudal direction beginning from the abdominal aorta at the origin of the inferior mesenteric artery. At bifurcations, the first axial section that did not contain any part of the "mother vessel" was described as the beginning of the "daughter vessel" (Figures 2.1). The T2w FSE, T2* GRE and T1w image series were reviewed for each patient to identify the points of bifurcation. The paired external iliac artery and vein were delineated inferiorly to the level where the inguinal ligament traversed the medial aspect of the artery. To ensure consistency among patients, segmentation of these structures was continued to the superior aspect of the femoral heads in all cases.

Vessel topology, course, segment length, and bifurcation location varies within and between individuals. Bifurcations of the iliac vessels on the right and left sides do demonstrate varying degrees of spatial asymmetry. Similarly, the vessel bifurcation angle may occur through a broad range. Vessel courses, lengths, curvatures, and branching angles will all impact upon object appreciation and segmentation accuracy. Given that this limitation exists within current clinical radiotherapy treatment planning, its presence within this study will not limit the clinical applicability of the results.
Figure 2.1 A to D: Cranial to caudal axial T2w FSE, T2* GRE and T1w images superior (A) to inferior (C and D) the bifurcation of the abdominal aorta. (Red arrow denotes the division of the aorta in the right and left common iliac arteries).
2.2.6 Lymph node nomenclature and segmentation

Following administration of ferumoxtran-10, lymph nodes were detected by a decrease in signal on the dual gradient-echo images. Lymph nodes were classified into anatomic groups according to the following definitions, as illustrated in Figure 2.1:

- **Distal para-aortic**: Adjacent to the aorta and IVC from the inferior mesenteric artery to the bifurcation of these vessels.
- **Sub-aortic/presacral**: Inferior to the sacral promontory over the mid-portion of the sacrum, medial to the sacral foramina and posterior to the peritoneum and the posterior rectal surface.
- **Common iliac**: Adjacent to the common iliac vessels from the aortic bifurcation to the branching of the internal iliac artery.
- **Internal iliac**: Along the medial aspect of the internal iliac vessels.
- **External iliac**: Adjacent to the external iliac vessels inferior to the origin of the deep inferior epigastric vessels, extending anterior to the iliopsoas muscle and posterior to include the obturator lymph nodes within the obturator fossa.

All visible lymph nodes in these regions were contoured regardless of size and whether or not they were judged to contain metastatic disease.

2.2.7 Musculoskeletal boundary nomenclature and segmentation

Musculoskeletal landmarks were identified for the sub-aortic/presacral and pelvic sidewall lymph nodes. The sub-aortic/presacral landmark was the anterior aspect of the fifth lumbar vertebrae and sacrum from the bifurcation of the aorta and IVC superiorly to
the inferior aspect of S3, and laterally to the sacral foramina. The pelvic sidewall landmark was the ilium/ischium and internal obturator muscle from the bifurcation of the common iliac vessels superiorly to the femoral canal inferiorly.

2.2.8 Measure of Proximity of Lymph Nodes to Vascular Segments

To generate a complete three-dimensional spatial representation of the lymphatic tissue in each nodal segment, individual lymph nodes were first divided into small subunits measuring 0.5 (x-axis) x 0.5 (y-axis) x 3 (z-axis) mm in size using an automated algorithm (Figure 2.2). Each subunit represented 0.75 mm³ of lymphatic tissue. Distances in 3-dimensions from the center of each nodal subunit to the closest vascular edge (arterial or venous) were then calculated and grouped by vascular segment. Sub-aortic/presacral lymph nodes and iliac lymph nodes along the lateral pelvic sidewall were also localized in relation to musculoskeletal landmarks in the same manner. Histograms were generated for each vascular segment to illustrate the spatial distribution of nodal tissue in relation to the vessels or bony landmarks. Summary statistics were derived from the histograms to determine the vascular expansions required in each segment to encompass 50%, 80%, 90%, 99% and 100% of the lymphatic tissue.
Figure 2.2: A three-dimensional surface rendering of the contoured pelvic lymph nodes identified using ferumoxtran-10 in relation to the pelvic vasculature for a typical patient. Arteries are indicated in red, veins in blue and lymph nodes in green.
Figure 2.3: A three-dimensional surface rendering of the contoured pelvic lymph nodes (a) and a schematic representation (b) of the method used to determine the spatial distribution of lymphatic tissue. The distance from the center of each lymph node volume unit (green) to the closest artery (red) and vein (blue) was calculated in three dimensions.
2.3 Results

2.3.1 Lymph node frequency, size and location

A total of 1670 lymph nodes defined by 5663 separate nodal contours were detected in the 55 patients. The median number of lymph nodes was 30 with a range of 5 to 62 (mean 30 and standard deviation 12.8). For those lymph nodes below the chosen radiographic size criterion for metastasis (>10 mm), the median short axis size was 4 mm, with a range between 2 and 10 mm (mean 3.7 mm and standard deviation 1.2 mm). The median total nodal volume was 6650.0 mm$^3$ (760.6-31946.9 mm$^3$) and mean 7532.0 mm$^3$ (standard deviation 5569.9 mm$^3$). Table 2.1 summarizes the frequency distribution of lymph nodes in each of the vascular segments. Nodes were relatively uniformly distributed in the common iliac, external iliac and internal iliac regions, but were less frequently detected in the sub-aortic/presacral region as well as the distal para-aortic. There were no significant differences in nodal frequency, size or volume between the left and right sides of the pelvis or between males and females.
Table 2.1: Pelvic lymph node frequency and distribution

<table>
<thead>
<tr>
<th>Vascular segment</th>
<th>Number of nodes (Median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Distal vena cava and para-aortic</td>
<td>1 (0-16)</td>
</tr>
<tr>
<td>Common iliac</td>
<td>7 (0-20)</td>
</tr>
<tr>
<td>External iliac</td>
<td>11 (1-19)</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>5 (0-15)</td>
</tr>
<tr>
<td>Sub-aortic/presacral</td>
<td>2 (0-6)</td>
</tr>
</tbody>
</table>
The main purpose of this study was to define lymph node target volumes for radiotherapy. It is unlikely, based on previous reports, that MR image enhancement with ferumoxtran-10 will allow lymph node metastases <3 mm in size to be detected reliably\textsuperscript{122, 124}. Therefore, radiotherapy target volumes will need to encompass all lymph nodes and not just those with imaging-evidence of metastatic disease. In addition, lymph nodes that were enlarged and at high risk of harboring metastatic disease by commonly accepted CT and MR size criteria (short axis diameter >10 mm) were excluded from the histogram analysis to avoid biasing the results. This resulted in the removal of 7 lymph nodes from the analysis in 4 of the study patients (4 left internal iliac, 1 right common iliac, 1 right external iliac and 1 right external iliac) and represents less than 0.5% of the nodes identified.

2.3.2 Quantitative distance histogram analysis of lymph node volume

Lymph nodes in each patient were analyzed as a function of distance from the closest vessel. Figure 2.3 depicts representative histograms from a typical patient for each of the vascular regions. The small branches of the internal iliac vasculature in the presacral region were not consistently visualized on MR. Therefore, the sub-aortic/presacral lymphatic tissue was related to the closest aspect of the anterior sacrum rather than the nearest visible vessel to avoid biasing the results towards unusually long distances. For the same reason, obturator and medial internal iliac lymphatic tissue along the pelvic sidewall was related to the pelvic sidewall as previously described (see Materials and Methods).
The spatial distribution of nodal tissue for the 55 eligible patients is summarized in Table 2.1. On average, 90% of the lymph nodes were detected within 8.7 mm of the nearest vessel in the distal para-aortic region, 7.3 mm in the common iliac region, 10.1 mm in the external iliac region and 12.1 mm in the internal iliac region. However, there was marked variability among patients. While the distribution of the nodal tissue along the blood vessels was noted to be anisotropic and varied along the length of each relevant vascular segment, isotropic (uniform) margins of vascular expansion were assessed with the intent of generating a practicable method of 3D nodal CTV delineation.

The small vessels associated with the sub-aortic/presacral and obturator lymph nodes were not consistently visualized and readily identifiable musculoskeletal landmarks were used instead. In the sub-aortic/presacral nodal region, the mean distance anterior to the sacrum to encompass 90% of the nodal tissue was 8.2 mm and, in the obturator region, the mean distance from the pelvic sidewall to encompass 90% of the nodal tissue was 7.9 mm.
Figure 2.4: Histograms for a typical patient illustrating the spatial distribution of nodal tissue in relation to the closest edge of the nearest artery or vein in each anatomic nodal region: a) distal para-aortic; b) common iliac; c) external iliac; d) internal iliac; e) pelvic side-wall f) the sub-aortic/presacral. Note the different Y-axis scales, reflecting differences in total lymphatic nodal volume from one region to another.
2.3.3 Nodal CTV for pelvic radiotherapy

The main objective of this study was to define clinically practicable rules for determining a lymph node CTV for patients receiving pelvic radiotherapy. The spatial distributions of lymph node tissue in relation to vascular or musculoskeletal landmarks summarized in Tables 2.2 and 2.3 suggest that different margins of expansion around these anatomic surrogates may be optimal in different anatomic regions. The rules for nodal CTV delineation were derived using 3D margins of expansion sufficient to cover 90% of the nodal tissue in 90% of patients. This definition was chosen as a compromise between nodal coverage and excessive normal tissue irradiation. The required margins of expansion around the distal para-aortic, common iliac, external iliac and internal iliac vessels were 12 mm, 10 mm, 9 mm and 10 mm respectively. In addition, a 22 mm medial expansion from the lateral pelvic sidewall, contiguous anteriorly and posteriorly with the expansions of the external and internal iliac vessels respectively, was required to encompass the obturator and medial internal iliac lymph nodes. A 12 mm expansion was required anterior to the sacrum from S1-S3, contiguous laterally with the expansion of the iliac vessels, to encompass the sub-aortic/presacral nodes. Figure 2.4 illustrates the use of these guidelines to devise a nodal CTV for pelvic radiotherapy. The anatomic expansions were trimmed to exclude bone, muscle and fascia, as well as bowel and bladder, because the space occupied by any of these tissues has a very low probability of also containing nodal tissue either at the time of imaging or at later times (data not shown).
Table 2.2: Spatial distribution of lymph node tissue in relation to the closest vessel in each vascular segment

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Distance of lymphatic tissue from nearest vessel (Mean for the entire cohort and range in individual patients, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Distal para-aortic</td>
<td>4.8 (2.4-11.5)</td>
</tr>
<tr>
<td>Common iliac</td>
<td>4.1 (2.1-10.5)</td>
</tr>
<tr>
<td>External iliac</td>
<td>5.2 (2.4-11.5)</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>7.0 (2.8-20.3)</td>
</tr>
<tr>
<td>Sub-aortic/pre-sacral*</td>
<td>15.5 (3.6-31.1)</td>
</tr>
</tbody>
</table>

Lymphatic tissue in each anatomic region was localized in relation to the closet artery or vein.
Table 2.3: Spatial distribution of lymph node tissue in relation to the closest vessel in each vascular segment and musculoskeletal land markers

<table>
<thead>
<tr>
<th>Percentile</th>
<th>50%</th>
<th>80%</th>
<th>90%</th>
<th>95%</th>
<th>99%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal para-aortic</td>
<td>4.6</td>
<td>7.0</td>
<td>8.2</td>
<td>8.8</td>
<td>9.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>(2.4-11.5)</td>
<td>(3.2-17.7)</td>
<td>(3.6-19.8)</td>
<td>(3.7-20.9)</td>
<td>(4.0-22.4)</td>
<td>(4.0-23.8)</td>
</tr>
<tr>
<td>Common iliac</td>
<td>4.0</td>
<td>6.1</td>
<td>7.2</td>
<td>8.2</td>
<td>9.6</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>(2.1-10.4)</td>
<td>(2.8-18.8)</td>
<td>(3.1-20.9)</td>
<td>(3.2-21.9)</td>
<td>(3.5-23.1)</td>
<td>(3.8-23.9)</td>
</tr>
<tr>
<td>External iliac</td>
<td>3.9</td>
<td>5.5</td>
<td>6.6</td>
<td>7.6</td>
<td>9.4</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>(2.4-5.5)</td>
<td>(3.1-7.8)</td>
<td>(3.4-10.1)</td>
<td>(3.7-12.3)</td>
<td>(4.2-15.0)</td>
<td>(4.2-17.8)</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>4.6</td>
<td>6.1</td>
<td>6.9</td>
<td>7.6</td>
<td>8.5</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>(2.8-8.4)</td>
<td>(3.3-11.0)</td>
<td>(3.4-13.5)</td>
<td>(3.4-14.5)</td>
<td>(3.4-15.6)</td>
<td>(3.4-16.6)</td>
</tr>
<tr>
<td>Sub-aortic/pre-sacral*</td>
<td>6.0</td>
<td>7.8</td>
<td>8.2</td>
<td>8.5</td>
<td>8.8</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>(3.0-10.3)</td>
<td>(3.5-15.1)</td>
<td>(3.8-16.5)</td>
<td>(4.2-17.2)</td>
<td>(4.2-17.8)</td>
<td>(4.2-18.2)</td>
</tr>
<tr>
<td>Obturator*</td>
<td>4.6</td>
<td>7.1</td>
<td>7.9</td>
<td>9.8</td>
<td>11.8</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>(2.6-7.2)</td>
<td>(3.4-12.2)</td>
<td>(3.6-18.9)</td>
<td>(3.9-19.8)</td>
<td>(4.3-21.1)</td>
<td>(4.3-22.7)</td>
</tr>
</tbody>
</table>

* Lymphatic tissue in each anatomic region was localized in relation to the closet artery or vein except in the sub-aortic/pre-sacral and obturator regions, where it was related to musculoskeletal land markers (see text).
2.4 Discussion

The lymphatic system is a prominent route of spread for many malignancies. Current imaging techniques are limited in their ability to reliably detect micro-metastases in lymph nodes. Therefore, radiation treatment of apparently normal lymph nodes is often advocated as a means of improving local disease control and improving survival in patients with endometrial, cervix, bladder, prostate and gastrointestinal cancer. The current evolution towards the use of high-precision IMRT to treat pelvic lymph nodes at risk of harboring occult disease demands accurate knowledge of the location of these nodes for target volume definition. This study provides a comprehensive description of the spatial distribution of pelvic lymph nodes derived from MR imaging and the use of a lymph node contrast agent in 55 patients. The results of this study demonstrate that: 1. ferumoxtran-10 subjectively enhances the capability of MR to detect pelvic lymph nodes, whether involved by cancer or not; 2. for CTV definition, appropriate margins of expansion around major pelvic vessels or musculoskeletal nodal surrogates vary according to anatomic regions; and 3. the margins of expansion that are required around these anatomic surrogates to reliably encompass high-risk nodes are larger than advocated in previous studies\textsuperscript{169}. A population-based model of pelvic lymphatic topography has been presented, along with a set of clinically-relevant rules for nodal target volume definition.

This study, in agreement with others, demonstrated that pelvic lymph nodes are generally situated close to vessels\textsuperscript{3}. This was particularly evident in the distal para-aortic, common iliac and external iliac regions, as illustrated in Figure 2.3 for a
representative patient and summarized for the entire cohort in Tables 2.2 and 2.3. However, nodal tissue in the sub-aortic/presacral and obturator regions was found at greater distance from the major arteries and veins. These nodes are associated with smaller branches of the iliac vessels that are too small to be visualized reliably using MR. Therefore, the major vessels alone do not provide sufficient surrogate information for lymph node CTV definition, and it was necessary to incorporate bony landmarks into our population-based model of nodal topography. The model suggests that 90% of the nodal tissue in 90% of patients will be encompassed by symmetrical three-dimensional expansions of the distal para-aortic (12 mm), common iliac (10 mm), external iliac (9 mm) and internal iliac (10 mm) vessels, drawn in continuity with a 12 mm expansion anterior to the sacrum and a 22 mm expansion medial to the pelvic sidewall, as shown in Figure 2.4. The union of the major artery and vein contours should be used to form the nodal CTV, and the resultant volume trimmed to exclude bone, muscle, fascia, bladder and bowel, consistent with other recommendations. While population-based models facilitate treatment planning, it is important to carefully review all relevant imaging for each patient and adapt the nodal CTV contours to encompass overt lymphadenopathy or nodal outliers.

There are at least two other descriptions of pelvic lymph node topography for radiotherapy treatment planning derived from MR imaging of ferumoxtran-10\textsuperscript{169,170}. Shih et al. mapped the center of mass of lymph nodes metastases in 18 men with prostate cancer to a standardized three-dimensional model using anatomic landmarks\textsuperscript{170}. A 2 cm uniform margin of expansion was found to encompass 94.5%
Figure 2.4: Pelvic nodal CTV at six cranio-caudal levels in the pelvis developed using our population-based model of lymph node tomography. The vascular, sacral and pelvic sidewall expansions were trimmed to exclude bone, muscle, fascia, bladder and bowel. (a) Distal para-aortic, (b) Bifurcation of the aorta and inferior vena cava, (c) Inferior to the bifurcation of the common iliac vessels, (d) Mid-pelvis (e) Cranial to the acetabulum, and (f) Mid-acetabulum.
of the lymph nodes thought to be at risk of harboring disease, assuming a fixed nodal
diameter of 1 cm. This margin of expansion probably is excessive based on our analysis
of the spatial distribution of lymphatic tissue in 55 patients, and would unnecessarily
limit the utility of IMRT to spare adjacent normal tissues. Taylor et al., in a series of 20
women with gynecologic tumors, found a 7 mm vessel expansion to be optimal provided
that the contours were manually modified using specified rules to include potential nodal
locations at greater distances from major arteries and veins\(^\text{169}\). Despite the apparent
difference in the proposed margins of expansion between this study and ours, the
derived nodal CTV's are actually quite similar: The manual contour modifications that
are implicit in the Taylor et al. CTV definition, in effect, further expand their 7 mm vessel
margins so that the final volumes are relatively coincident. Our approach may be less
time consuming, and less prone to error, because less revision is required for individual
cases.

The greatest limitation of this and previous studies is the ability of MR imaging with
ferumoxtran-10 to identify small lymph nodes <3 mm in size, and microscopic lymph
node metastases\(^\text{124, 174}\). The nodes comprising the presacral and internal iliac groups
are likely underrepresented by virtue of their size. Taylor et al. reported the frequency of
lymph node contours and a summation of consecutive contours through successive
axial images is required to determine the lymph node numbers identified for each lymph
node group specified\(^\text{169}\). Within their 20 patient series, a mean of 0.4 (0-2) and 7.2 (1-
22) lymph node contours were identified for the presacral and internal iliac lymph node
groups respectively. In comparison, a median 2 (0-6) and 5 (0-15) presacral/subaortic
and internal iliac lymph nodes were identified in our 55 patient series. Recognizing that
the presacral lymph node group in our series included subaortic lymph nodes and that nodes within each group may lie across more than one adjacent axial plane, there would appear to be an equivalent or greater number of lymph nodes identified in our series with a larger sample size. Our model for nodal CTV definition was derived from the spatial distribution of visible lymph nodes, on the assumption that these would be representative of all nodes at risk of harboring disease. It is possible that there are small lymph nodes at greater distances from the major vessels that were not visualized using this approach. However, these are likely to represent a small proportion of the total nodal volume in each anatomic region and would not substantially alter the proposed margins of expansion, which were defined to encompass 90% of the lymphatic tissue in 90% of patients. Ferumoxtran-10, while lowering the MR threshold for the detection of subclinical disease, does not enhance the sensitivity enough to reliably detect microscopic metastases\textsuperscript{174}. This currently limits the utility of this approach for defining patient-specific radiotherapy volumes that target only regions of known tumor. However, innovative cellular MR techniques using magnetic nanoparticles have been shown in preclinical studies to detect as few as 100 malignant cells in lymph nodes, and hold great promise for the future\textsuperscript{175}. 

2.6 Conclusion

In conclusion, the use of MR lymphography provides an objective, three-dimensional spatial description of relevant pelvic lymph nodes in relation to easily visualized anatomic landmarks. Radial 3D margins of expansion around the major pelvic vessels, with the inclusion of separate margins for the sacral and medial external and internal iliac lymph nodes, can be used to develop a population-based nodal CTV for radiotherapy treatment planning. The use of this model in clinical practice will assure a high probability of encompassing most of the nodal tissue at risk of harboring metastases in most patients, while minimizing the dose to normal tissues.
Chapter 3. Inguinal Lymph Node Topography for Radiotherapy Treatment Planning from Contrast-Enhanced MR Imaging

3.1 Introduction

Primary and adjuvant inguinal radiotherapy is employed in the management of genitourinary, gynecologic and gastrointestinal malignancies. Intensity modulated radiotherapy may permit the delivery of an escalated dose to the lymph nodes at risk of occult metastatic spread while potentially reducing the probability of toxicity such as lymphedema and fibrosis of normal tissues.

Adequate delineation of a nodal clinical target volume (CTV) for inguinal lymph node irradiation is predicated upon sound knowledge of lymphatic anatomy. Unfortunately, the location and depth of the inguinal lymph nodes has not been well defined and inguinal nodal radiotherapy volumes are traditionally related to anatomic surrogates such as bony landmarks or the femoral vasculature\textsuperscript{176, 177}. A potential inability to accurately define and encompass the inguinal lymphatic CTV may result in the delivery of an inadequate dose and a geographic miss resulting in a failure to eradicate clinically evident or subclinical disease\textsuperscript{178}. Koh \textit{et al.} demonstrated the importance of adequately defining the depth of the inguinal lymph nodes in a re-analysis of patients with inguinal nodal recurrence following treatment for vulvar carcinoma\textsuperscript{179}. The use of an absolute depth to represent the position of the inguinal nodes without regard to an individual patient’s weight and body habitus resulted in a failure to adequately encompass the lymph node CTV within the high dose radiation volume and subsequent high risk of
inguinal node failures. From that analysis, an average patient would require a prescription depth of 6.0 cm to adequately encompass the inguinal lymphatics. However as demonstrated by the significant range observed (minimum mean 6.1 cm and maximum mean 17.3 cm), this depth would need to be modified for a given individual to respect patient specific anatomic factors. A poor understanding of inguinal lymph node target location relative to other anatomic landmarks affected this study\textsuperscript{178, 180, 181}. A related follow-up study analyzing 31 women with cervical, vaginal or vulvar carcinomas revealed the mean depth of the most superficial inguinal lymph node to be 2.6 cm and the mean depth of the deepest to be 5.8 cm\textsuperscript{181}. These depths were dependent on body habitus and further support the need for a patient specific plan as opposed to an arbitrary prescription depth.

CT based treatment planning has the ability to identify the vessels within an individual patient avoiding the potential for over or under estimating the depth of the femoral vessels. However, CT lacks sufficient soft tissue resolution to depict the small clinically normal lymph nodes of interest and little is known about the precise anatomic location of the inguinal lymph nodes relative to the femoral vessels and other adjacent anatomic structures. While knowledge of nodal topography is still incomplete, it has been known for some time that the spatial relationship between the lymphatics and the vasculature is one of high anatomic reproducibility and fidelity\textsuperscript{7, 19, 55}. Wang et al. plotted the topographic distribution of clinically palpable gross inguinal lymphadenopathy on a representative fluoroscopic simulation film to derive field boundaries to encompass the inguinal lymphatics\textsuperscript{176}. While this may be suitable for two-dimensional planning, it does not adequately convey sufficient information to generate an acceptable patient specific
three-dimensional radiotherapy treatment plan. For conformal and intensity modulated radiotherapy no validated rationale for the generation of an inguinal nodal clinical target volume is yet available.

The objective of this study was to develop a robust and generalizable CTV definition for inguinal lymph node irradiation based on an isotropic expansion of vascular anatomy to encompass nodal frequency and topography using MR lymphography with ferumoxtran-10 enhanced magnetic resonance images of the lower pelvic and inguinal region. Based on an analysis of this data, we provide a practical working CTV definition that can be applied to patients with gynecologic, genitourinary or gastrointestinal tumors who are deemed to be at high risk of harboring occult inguinal lymph node metastases.

3.2 Materials and Methods

This was a single center pilot study eligible to patients with histologically confirmed endometrial, cervix, prostate (cT1-cT3) or bladder cancer with no distant metastases. To ensure uniformity of the sample and to control for artifacts and lymphadenopathy, all subjects were screened to exclude: men with histologically confirmed carcinoma of the prostate and a PSA < or = 4.0 or > 100; evidence of distant metastasis (M1); radical surgery or prior cryosurgery for carcinoma of the prostate, previous radiation, hormonal manipulation or chemotherapy; patients with biopsy-proven lymph node involvement; women with histologically confirmed carcinoma of the endometrium or cervix who have undergone recent pelvic surgery with the exclusion of colposcopy, dilatation and curettage or biopsy; men or women with histologically confirmed carcinoma of the
bladder who have undergone recent pelvic surgery with the exclusion of TURBT; previous or concurrent cancers other than superficial basal or squamous cell skin carcinoma unless disease free for at least 5 years; or a hip prosthesis. MR imaging remained uniform with no changes in equipment or technique over the duration of the study period.

3.2.1 Patient characteristics

From August 2004 to June 2006, 30 patients were prospectively enrolled in this study with biopsy proven primary carcinoma of the prostate (20), bladder (5), endometrium (3) or cervix (2). Patients who had undergone prior chemotherapy, radiotherapy or pelvic/inguinal lymph node dissection or hernia surgery were excluded. None of the patients analyzed for this study had clinically or radiographically detectable inguinal nodal metastases. The median age was 64 years (range 39-82). There were 25 men and 5 women. All patients were imaged prior to undergoing therapy for their cancer. The local Research Ethics Board approved the study and all of the patients who participated provided written informed consent.

3.2.2 MR imaging

MR imaging was carried out using a 1.5 T magnet (GE EXCITE 1.5 T, Waukesha, WI, USA) with a four-channel pelvic phased-array coil. Patients were instructed to take nothing by mouth four hours prior to the study.

Axial imaging sequences were obtained at 3 mm intervals through the pelvis before and 24-36 hours after contrast administration. Pulse sequences used consisted of a T2w
FSE (TR/TE 6500/80; field of view 24-28 cm), and a T2*w dual echo GRE (TR/TE1/TE2 1400/14/21ms; field of view 26-28 cm). Motion artifacts from peristalsis were minimized with the administration of hyoscine-N-butylbromide (Buscopan) 30 mg or glucagon 1mg i.v. or i.m. prior to each of the MR imaging sessions.

3.2.3 Ferumoxtran-10

Ferumoxtran-10 (Combidex, AMAG Pharmaceuticals; Lexington, MA) was reconstituted from a lyophilized powder. A dose of 2.6 mg Fe/kg was dissolved in 100 ml of normal saline (0.9% NaCl) and infused intravenously.

3.2.4 Image analysis

Image analysis was performed on archived DICOM images using a PACS radiology workstation (OsiriX image processing software, Version 2.7.5; OsiriX Foundation, Geneva, Switzerland) and 3D modeling and image processing software (3D-DOCTOR®: Able Software Corp., Lexington, MA). A single observer (R.D.) manually delineated the lymph nodes, inguinal and pelvic vasculature, as well as the skin and musculoskeletal landmarks. Lymph node size evaluation was undertaken using the T2w FSE image series as opposed to the T2*w dual echo GRE which is subject to USPIO induced susceptibility artifacts. To verify the identification and anatomic accuracy of the manual operator (R.D.) defined regions of interest (vascular segments and lymph nodes) an expert abdominal-pelvic radiologist (M.H.) reviewed 10 (33%) of the image datasets. No noted variation between the observers was apparent with regards to either nodal identification and delineation or vessel segment identification. Points defining the edge of each distinct vascular and nodal element were identified in the x- and y- planes for each axial slice (z-plane).
3.2.5 Vascular segmentation

All image sets were reviewed with partitioning of the image data to define the vascular and lymphatic structures of interest. Manual tracing of the vessel walls was undertaken using continuous sampling with vertices recorded along the path length. A closed spline curve was then generated by connection of the vertices. Object specific boundaries on each axial slice were integrated through the series to define a three-dimensional volume. All points \((x, y, \text{ and } z)\) at the boundary edge and those pixels enclosed by these points were considered to be part of the object. Those points and pixels not part of a target structure were considered to be background. Window width and window level were standardized to ensure optimization of contrast and brightness.

Segmentation of the inguinal arterial and venous system was performed in a cranio-caudal direction beginning from the external iliac artery and vein at the level where the inguinal ligament traversed the medial aspect of the artery. At bifurcations, the first section that did not contain any part of the "mother vessel" was described as the beginning of the "daughter vessel ". The T2w FSE, T2* GRE and T1w image series were reviewed for each patient to identify the points of bifurcation. Segmentation of the venous system was performed in an analogous manner. The vessels of interest (femoral artery and vein, superficial circumflex iliac vein, great saphenous vein, superficial external pudendal vein and the superficial epigastric vein) were delineated from their origin at the point of the passage of the external iliac artery and vein under the inguinal ligament superiorly and distally to the inferior border of Scarpa’s triangle defined by the adductor longus and sartorius muscles on both the right and left sides\(^7\).
Vessel topology, course, segment length, and bifurcation location varies within and between individuals. Bifurcations of the iliac vessels on the right and left sides do demonstrate varying degrees of spatial asymmetry. Similarly, the vessel bifurcation angle may occur through a broad range. Vessel courses, lengths, curvatures, and branching angles will all impact upon object appreciation and segmentation accuracy. Given that this limitation exists within current clinical radiotherapy treatment planning, its presence within this study will not limit the clinical applicability of the results.

### 3.2.6 Lymph node nomenclature and segmentation

After administration of ferumoxtran-10, lymph nodes were identified by a decrease in signal on the gradient-echo images, which was more marked at the longer TE. Localization was aided by combined review with T2 images. The visualized lymph nodes were identified and divided into right and left sides as illustrated in Figure 3.1. All of the lymph nodes lying within anatomic volume defined by the inguinal ligament, the adductor longus muscle and the sartorius muscle, were segmented as separate structures.

Prior to analyzing the patient data, our approach was validated using the photomicrographs of the adult human male (Visible Human Male – VHM) from the Visible Human Project, National Library of Medicine\[20\]. The VHM dataset is comprised of high resolution, 1 mm, anatomic, axial images, and allows lymph nodes as small as 1 or 2 mm in size to be identified reliably. Lymph nodes in this size range may not be seen even with ferumoxtran-10 enhanced MR images of the study subjects, and the VHM was therefore used to assure that the nodal distribution maps and the nodal CTV
definition derived from the patient MR image sets were truly representative of the nodal anatomy.

3.2.7 Quantitative distance histogram analysis of lymph node volume

The x-y-z coordinates of the segmented structures (vascular and lymphatic) were used to compute their spatial relationships. The boundary descriptors for the pelvic vasculature and lymphatics were compiled in one data file for each study subject and analyzed. Each lymph node in every image set was divided into nodal volume elements measuring 0.5 (x-axis) x 0.5 (y-axis) x 3 (z-axis) mm in dimension using an automated algorithm in order to generate a complete 3D spatial representation of all of the lymphatic tissue. Paired 3D distances were determined from the centre of each nodal volume element (representing 0.75 mm$^3$ of lymphatic tissue) to the closest vascular edge (arterial or venous) for each vascular segment across all axial planes (Figure 3.2).
Figure 3.1: (A) A three-dimensional image rendering of the contoured lymph nodes, vasculature and bony anatomy derived from the VHM with a representative image adjacent. (B) Three-dimensional rendering of pelvic and inguinal vasculature (1 inguinal ligament, 2 sartorius muscle, 3 adductor longus muscle, 4 femoral artery and vein, 5 superficial circumflex iliac vein, 6 great saphenous vein, 7 inferior epigastric vein and the 8 external pudendal vein) and (C) the corresponding lymph nodes.
Anisotropic and isotropic histograms describing the distribution of distances from the centre of each nodal volume element to the nearest vessel edge for varying bin sizes were generated for each vascular segment to illustrate the spatial distribution of nodal tissue in relation to the associated vessels. Scatter plot displays of the nodal volume elements were plotted on a Cartesian coordinate system where the intersection of the abscissa and ordinate (origin) was defined as the collapse surface of the vessels comprising the vascular segments of interest. Summary statistics were derived from the histograms to determine the vascular expansions required in each vascular segment to encompass 50%, 80%, 90%, 95%, 99% and 100% of the lymphatic tissue. From this, uniform (isotropic) vascular margins of expansion were generated. These margins were then applied to the vessels to produce an inguinal nodal CTV delimited by the adjacent musculature, body of the pubis and skin.

3.3 Results

On each of the 1 mm axial macrotomal sections of the Visible Human Male dataset, the inguino-femoral region bounded by the inguinal ligament superiorly, medially, and laterally by the adductor longus and sartorius muscles were delineated. One hundred and sixty one sections (16.1 cm) encompassed this volume. On the right, 8 discrete inguinal lymph nodes were identified with a range in axial size of 3 to 16 mm (mean 6) and on the left, 17 separate lymph nodes were identified with a range in greatest axial size of 2 to 14 mm (mean 5) (Figure 3.1). The total volume of nodal tissue on the right was 2690.3 mm$^3$ and 3114 mm$^3$ on the left.
Figure 3.2: (A) A three-dimensional surface rendering of the contoured left inguinal lymph nodes with a schematic representation of the method used to determine the spatial distribution of lymphatic tissue inset. (B) The distance from the center of each lymph node volume unit (green) to the closest artery (red) and vein (blue) was calculated in three dimensions.
Following delineation of the lymph nodes in the VHM, the right and left femoral artery and vein, superficial circumflex iliac vein, great saphenous vein, superficial external pudendal vein and the inferior epigastric vein were identified and contoured. The distances from each vessel to the lymph nodes in three-dimensions were analyzed individually for a given vessel and in combination with the other vessels to determine which vessel(s) were most closely related to the adjacent lymph nodes (Table 3.1). The femoral artery and vein, superficial circumflex iliac vein and the great saphenous vein had the most clinically relevant associations to the adjacent lymph nodes while the superficial external pudendal vein and the inferior epigastric vein had the least relevant ones (data not shown).
Table 3.1: Vessel combinations and the spatial distribution of lymph node tissue in relation to the closest vascular segment

<table>
<thead>
<tr>
<th>Vessels</th>
<th>50% of LN Vol.</th>
<th>80% of LN Vol.</th>
<th>90% of LN Vol.</th>
<th>95% of LN Vol.</th>
<th>99% of LN Vol.</th>
<th>100% of LN Vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F AV</td>
<td>13.9</td>
<td>17.1</td>
<td>18.8</td>
<td>20.4</td>
<td>25.0</td>
<td>28.0</td>
</tr>
<tr>
<td>F AV GS V</td>
<td>11.1</td>
<td>15.3</td>
<td>17.6</td>
<td>20.1</td>
<td>25.0</td>
<td>28.0</td>
</tr>
<tr>
<td>F AV SCI V</td>
<td>9.5</td>
<td>15.1</td>
<td>16.9</td>
<td>18.2</td>
<td>20.0</td>
<td>22.8</td>
</tr>
<tr>
<td>F AV GS V SCI V</td>
<td>7.4</td>
<td>10.8</td>
<td>12.7</td>
<td>14.3</td>
<td>16.6</td>
<td>22.3</td>
</tr>
</tbody>
</table>

F AV: femoral artery and vein; GS V: Great Saphenous Vein; SCI V: Superficial Circumflex Iliac Vein.
The high resolution (4096 x 2048 x 24 bits) color images of the axial anatomic images of the VHM afford an opportunity for a detailed study of lymphovascular anatomy\textsuperscript{20}. Current clinically available MR and CT imaging is not able to provide this level of anatomic detail and resolution. To approximate what may be imaged in current clinical care, those lymph node contours, which were 3 mm or less in diameter were censored and the remaining lymph nodes were analyzed to yield distances of 15.2, 18.0, 19.6, 21.4, 25.2 and 27.7 mm to encompass 50\%, 80\%, 90\%, 95\%, 99\% and 100\% of the inguinal lymph nodes. Censoring those lymph nodes, which were greater than 3 mm in diameter, resulted in distances of 11.7, 14.7, 16.6, 18.3, 23.7 and 28.0 mm. The analysis of this dataset demonstrates the feasibility of using identifiable vascular surrogates to define nodal positions and those lymph nodes which may be at the threshold of detection or fall below it do not appear to adversely impact upon the lymph node to vessel distance data obtained. Those nodes that are larger than 3 mm in diameter (detectable with the current spatial resolution of clinically available MR scanners) are related closely enough to those less than or equal to 3 mm so that the smaller nodes would be encompassed by the values defined using the larger nodes.

Based upon the findings from the VHM analysis, the ferumoxtran-10 data were analyzed (Figure 3.3). For the thirty patients accrued from August 2004 to June 2006, a mean of 8 and median of 8 inguinal lymph nodes were identified within the volume encompassing Scarpa’s triangle on both the right and left side (Range: 3-16 right and 2-13 left) with a mean and median depth of 26.7 and 26.4 mm (9.4-49.5) on the right and 27.7 and 27.3 mm (8.1-52.9) on the left. There was no significant difference in the number and distribution of inguinal lymph nodes between the left and right sides. The
mean and median short axis diameters of the lymph nodes were 5.7 and 6 mm (range 3-12). Tables 3.2 to 3.4 depict the distance of the nodal volume elements from the closest vessel edge using both isotropic and anisotropic analyses. The effect of the inclusion of the superficial circumflex iliac vein upon the distribution of the nodal volume elements in relationship to the surface of the nearest vessel is illustrated in Figure 3.4. With the inclusion of the superficial circumflex iliac vein, a reduction in the isotropic margin of vascular expansion is achieved by better capturing the true anatomical spatial relationships of the lymph nodes to the corresponding vessel. Using only the femoral artery and vein, the nodal tissue is distributed in a hemispheric fashion anterior to the vessels with 90% of this tissue lying within 27.7 mm of the vessels. The congruence in the distribution on the scatter plot with the adjacent anatomic rendering demonstrates the absence of visible lymph nodes posterior to the femoral vessels (Figure 3.4).
Figure 3.3: Histograms derived from the Visible Human Male illustrating the spatial proximity of nodal tissue in relation to the closest edge of the nearest artery or vein for: a) the femoral artery and vein; b) femoral artery and vein and great saphenous vein; c) femoral artery and vein and superficial circumflex iliac vein and d) femoral artery and vein, great saphenous vein and superficial circumflex iliac vein.
Table 3.2: Spatial distribution of lymph node tissue in relation to the closest vascular segment (Femoral Artery and Vein) derived from the 30 patient series

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Distance of lymphatic tissue in mm from nearest vessel to encompass a given percentage of the identified lymph node (LN) volume (Vol.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% of LN Vol.</td>
</tr>
<tr>
<td>10th</td>
<td>9.7</td>
</tr>
<tr>
<td>20th</td>
<td>10.2</td>
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<tr>
<td>30th</td>
<td>10.8</td>
</tr>
<tr>
<td>40th</td>
<td>12.1</td>
</tr>
<tr>
<td>50th</td>
<td>13.5</td>
</tr>
<tr>
<td>60th</td>
<td>14.2</td>
</tr>
<tr>
<td>70th</td>
<td>15.4</td>
</tr>
<tr>
<td>80th</td>
<td>16.9</td>
</tr>
<tr>
<td>90th</td>
<td>18.8</td>
</tr>
<tr>
<td>100th</td>
<td>21.4</td>
</tr>
</tbody>
</table>
Table 3.3: Spatial distribution of lymph node tissue in relation to the closest vascular segment (Femoral Artery and Vein and Superficial Circumflex Iliac Vein) derived from the 30 patient series

<table>
<thead>
<tr>
<th>Percentile</th>
<th>50% of LN Vol.</th>
<th>80% of LN Vol.</th>
<th>90% of LN Vol.</th>
<th>95% of LN Vol.</th>
<th>99% of LN Vol.</th>
<th>100% of LN Vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10th</td>
<td>6.4</td>
<td>9.4</td>
<td>10.9</td>
<td>12.2</td>
<td>14.1</td>
<td>14.8</td>
</tr>
<tr>
<td>20th</td>
<td>6.8</td>
<td>9.9</td>
<td>11.9</td>
<td>14.4</td>
<td>16.2</td>
<td>17.5</td>
</tr>
<tr>
<td>30th</td>
<td>7.8</td>
<td>11.7</td>
<td>13.4</td>
<td>15.3</td>
<td>17.9</td>
<td>19.0</td>
</tr>
<tr>
<td>40th</td>
<td>8.2</td>
<td>12.7</td>
<td>14.9</td>
<td>17.1</td>
<td>19.3</td>
<td>20.7</td>
</tr>
<tr>
<td>50th</td>
<td>8.7</td>
<td>13.7</td>
<td>16.0</td>
<td>17.6</td>
<td>21.1</td>
<td>23.3</td>
</tr>
<tr>
<td>60th</td>
<td>9.1</td>
<td>14.8</td>
<td>17.1</td>
<td>20.0</td>
<td>22.2</td>
<td>24.3</td>
</tr>
<tr>
<td>70th</td>
<td>10.2</td>
<td>15.6</td>
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</tr>
<tr>
<td>80th</td>
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<td>17.0</td>
<td>19.2</td>
<td>21.9</td>
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<td>26.6</td>
</tr>
<tr>
<td>90th</td>
<td>11.7</td>
<td>17.8</td>
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<td>22.5</td>
<td>26.9</td>
<td>28.7</td>
</tr>
<tr>
<td>100th</td>
<td>13.7</td>
<td>18.9</td>
<td>21.0</td>
<td>22.7</td>
<td>37.7</td>
<td>40.4</td>
</tr>
</tbody>
</table>
Table 3.4: Anisotropic analysis of the spatial distribution of lymph node tissue in relation to the closest vascular segment derived from the 30 patient series

<table>
<thead>
<tr>
<th>Vessels</th>
<th>ANT MED</th>
<th>ANT</th>
<th>ANT LAT</th>
<th>POST LAT</th>
<th>POST</th>
<th>POST MED</th>
</tr>
</thead>
<tbody>
<tr>
<td>F AV</td>
<td>21.1</td>
<td>24.3</td>
<td>32.1</td>
<td>0</td>
<td>0</td>
<td>10.1</td>
</tr>
<tr>
<td>SCI V</td>
<td>15.3</td>
<td>20.2</td>
<td>19.7</td>
<td>7.9</td>
<td>8.7</td>
<td>13.1</td>
</tr>
</tbody>
</table>

F AV: femoral artery and vein; SCI V: Superficial Circumflex Iliac Vein.
Inclusion of the superficial circumflex iliac vein, which does have lymph nodes related to its posterior aspect, results in a significant change in the distribution of the lymph node volume elements around the vessels’ surfaces. While the bulk of the elements are still situated antero-laterally, points are now apparent posterior to the surface effecting a partial shift to a more circumferential distribution closer to the vessel surface (coordinate (0,0) on the scatter plot). The radial space around the vessel surfaces was divided into six equal parts and analysis of the distribution of the lymph node volume elements was undertaken to determine the anisotropic distribution of the lymph nodes. Table 3.4 depicts the margin of expansion to encompass 90% of the lymph nodes in 90% of the patients for each sextant.
Figure 3.4: (A). A frontal view of a three-dimensional surface rendering for a typical patient with the left femoral artery and vein and inguinal lymph nodes delineated and (B) the corresponding scatter plot distribution of the lymph node volume elements in relation to the point collapse surface of the femoral artery and vein. (C) A frontal view with the left femoral artery and vein and superficial circumflex iliac vein delineated and (D) the corresponding scatter plot distribution of the lymph node volume elements in relation to the point collapse surface of these vascular segments. Note the antero-radial distribution of the lymph node volume elements in relationship to the femoral vessels and the effect of the inclusion of the superficial circumflex iliac vein on the distribution.
3.3.1 Nodal CTV for adjuvant inguinal radiotherapy

The main objective of this study was to define a set of practical clinical rules for determining a lymph node CTV in relation to easily visualized anatomic surrogates of lymph node position. The margins of expansion to define the inguinal nodal CTV were based on coverage of 90% of the nodal tissue in 90% of patients and represent a compromise between nodal coverage and the avoidance of excessive normal tissue irradiation (Figure 3.5)\textsuperscript{183}.

An inguinal nodal CTV may be delineated by:

1. Segmenting the femoral artery and vein within Scarpa’s triangle;

2. Segmenting the superficial circumflex iliac vein within Scarpa’s triangle if a more refined nodal CTV with greater potential sparing of adjacent tissues is desired;

3. Segment the adjacent anatomic land markers defining Scarpa’s triangle;

4. Expand the vessel contours by 3 cm if only the femoral vessels are used or 2 cm if both the femoral and circumflex vessels are used;

5. Revise or delimit the expanded volume to exclude the adjacent musculature and those contours extending inferior to the region within which the sartorius muscle crosses the adductor longus muscle; and

6. Visually inspect the volumes to ensure inclusion of any potential enlarged lymph nodes or outliers.
Figure 3.5: Inguinal nodal clinical target volume at twelve cranio-caudal levels in the groin developed using our population-based model of lymph node topography. The vascular expansions were trimmed to exclude bone, muscle, and skin. Legend: Femoral arteries – red; Femoral vein – dark blue; Superficial circumflex iliac vein – light blue; Sartorius muscle – brown; Adductor longus muscle – khaki and lymph nodes – green.
3.4 Discussion

In spite of improvements in anatomic imaging and the use of novel radiologic contrast agents, our knowledge of lymphatic anatomy and ability to identify lymph nodes in vivo requires further improvement to more accurately identify occult nodal metastases and delineate patient specific patterns of loco-regional spread. The inguinal lymphatics are sites of potential spread for some gynecologic, genitourinary and gastrointestinal malignancies. With the use of existing anatomic and surgical data to provide parameters for a model, imaging information can be employed to generate a three-dimensional probability atlas for nodal location in relation to adjacent vessels and other anatomic structures. This model provides a means of defining a class solution for radiotherapy treatment volume delineation.

The inguinal lymph nodes are situated inferior to the inguinal ligament and are divided into superficial and deep components. The superior superficial inguinal nodes are distributed along the line of the inguinal ligament and lie anterior to the femoral fascia and deep to the subcutaneous fascia while the superficial inferior nodes are situated vertically along the proximal aspect of the great saphenous vein prior to its union with the femoral vein at the fossa ovalis (Figure 3.1). The number of superficial nodes varies with a range of 4 to 13 reported in the literature\(^7\). The deep inguinal lymph nodes are situated posterior to the fascia lata within the fossa ovalis (fossa ileopectinea) and lie medial to the femoral vein within the femoral canal. Typically 1-3 lymph nodes are identified within this region\(^7,49\).
Non-lymphatic anatomic structures, which can be related to the boundaries of the inguinal region and to the inguinal nodes, are of benefit in defining and refining a nodal CTV. In an attempt to minimize the morbidity of inguinal lymph node dissections, several studies have assessed the inferior, lateral, and superior extents of the deep and superficial lymph nodes. Borgno et al. reviewed the distribution of the deep inguinal lymphatics and noted that 33% of these nodes were situated inferior to the junction of the saphenous vein with the femoral vein and all of the nodes fell within the boundaries defined by the fossa ovalis. No lymph nodes were identified inferior to the most distal margin of the fossa ovalis. Additionally, no deep inguinal lymph nodes were identified lateral to the femoral artery. The superficial inguinal lymphatics have been studied in order to limit the lateral extent of the surgical field required for an inguinal lymph node dissection. These nodes lie within the superior aspect of Scarpa's triangle and are closely related to the superficial circumflex iliac vasculature. As a consequence of this close relationship, the lymph nodes are not typically found lateral to the medial aspect of the sartorius muscle superior to the point at which it crosses the lateral aspect of the inguinal ligament (Figures 3.1 and 3.2). Similarly, the posterior most extension of the inguinal lymph nodes have been shown not to extend beyond the point at which the sartorius muscle crosses the adductor longus muscle in the anatomic position (Figures 3.1 and 3.5).

The smallest short axis distance of the lymph nodes delineated in the VHM dataset was 2 mm. While this is smaller than the slice thickness (3 mm) used in this study and raises concerns about identifying these lymph nodes using MRI, the close relationship of these lymph nodes to the adjacent femoral vessels would result in them being encompassed in a nodal CTV generated using a margin of expansion derived on the basis of larger
visible nodes around these vessels. The adjacent lymph nodes which are of a larger size than the slice thickness used and are apparent on the MRI images can help to define the outer boundaries for an anatomic envelope to encompass the inguinal nodal basin within which the smaller (<3 mm) lymph nodes may reside.

The lymphatic system arises embryologically from the venous system with a strong anatomic and spatial relationship between the two. The topographic distribution of the lymph nodes is influenced by the adjacent nonvascular anatomy, which influences the spatial distribution of the lymph nodes in relation to the adjacent vessels. While isotropic margins of vascular expansion afford a sufficiently accurate and clinically practical means of lymph node CTV delineation, they are an over-simplification. The anisotropic distribution reflects the impact of the musculature, bony and integumentary anatomy on the location of the lymph nodes and shows their close association with the vessels. A dynamic adaptive margin of expansion to account for variations in nodal distribution through a consecutive series of axial planes would be required to fully exploit these anatomic relationships for clinical use. An adequate approximation for this can be obtained with an isotropic margin of expansion limited by adjacent structures. By delimiting the isotropic nodal expansion to avoid inclusion of the muscles surrounding the inguinal region the superior to inferior changes can be accounted for. With the aid of magnetic resonance lymphography to facilitate identification of the lymph nodes, the spatial relationship to the adjacent vessels has been determined and the expansions around the vessels necessary to encompass a specified percentage of these nodes have been quantified (Tables 3.1 and 3.2). This knowledge may be useful in radiographic staging and in surgical as well as radiotherapy treatment planning to facilitate nodal localization.
The use of conformal and intensity modulated radiotherapy enables sparing of adjacent normal tissues. This potential can be further optimized by using the adjacent musculature to limit an isotropic margin of expansion to produce a nodal CTV that encompasses all of the inguinal lymph nodes while eliminating the inclusion of adjacent normal structures within the CTV (Figure 3.5). While further studies are required to better delineate nodal patterns of failure, the nodal CTV generated using a modified 2 cm isotropic margin of expansion around the femoral artery, vein and the superficial circumflex vein provides a volume, which is consistent with that proposed by others while facilitating the potential for normal tissue avoidance 176, 188.

3.5 Conclusion

In conclusion, the use of MR lymphography has facilitated the construction of an objective, three-dimensional spatial description of relevant inguinal lymph nodes in relation to easily visualized anatomic landmarks. Radial 3D margins of expansion around the femoral artery, vein and the superficial circumflex iliac vein were used to develop a population-based inguinal nodal CTV for radiotherapy treatment planning. The use of this model in clinical practice will assure a high probability of encompassing most of the nodal tissue at risk of harboring metastases in most patients, while minimizing the dose to normal tissues.
Chapter 4. General Discussion

Prior to the development of lymphography, no clinically practical means of lymph node delineation for radiotherapy treatment was available. With the entry of oil-based lymphography into clinical practice, lymphograms were used to identify lymph nodes at high risk of harboring metastatic disease, which advanced the treatment of genitourinary, gynecologic, gastrointestinal and other malignancies. However, the sensitivity of clinical lymphography for the detection of microscopic disease is markedly inferior to surgical dissection and histologic examination. Non-contrast and to a significant extent contrast-enhanced x-ray radiographs of this era were unable to fully visualize relevant soft tissue anatomy including the pelvic organs, the vasculature as well as the lymphatics. Therefore, target localization for radiotherapy treatment planning was incomplete and imprecise, necessitating that large volumes of normal tissues be treated to avoid missing regions of disease. In the absence of a clinically feasible means for accurate vascular or lymphatic identification, the wealth of anatomic information obtained up to this point in time could not be adequately translated to the two-dimensional treatment planning radiographs nor could it be leveraged in the routine provision of clinical care.

The development and use of non-contrast and contrast enhanced CT and MRI and the availability of multi-planar cross-sectional imaging has enabled three-dimensional radiotherapy treatment planning. Multi-slice imaging with CT and MRI are increasingly able to generate three-dimensional visualizations of greater and greater anatomic detail. While there has been an improvement in the level of resolution that can be appreciated
with these modalities, they remain sub-optimal in the identification of small lymph nodes in the pelvis and groin as well as in the detection of microscopic lymph node metastases\textsuperscript{201, 202}. Functional imaging with PET scanning has the potential to improve the detection of clinically occult lymph node metastases to some degree but remains insensitive to micrometastatic disease\textsuperscript{203}. With further improvements in each of these modalities, the identification of anatomical features below the current limits of resolution will become a reality and rival the detailed anatomic information that can be obtained through surgical dissection. Similarly, the continued development of novel lymphotrophic contrast agents augurs a new era of lymphatic target definition. Systems of anatomic knowledge management will become increasingly important to ensure that the emerging information is utilized to its maximal potential for radiotherapy treatment planning as well as other endeavors. Chapters 2 and 3 outline the most comprehensive quantitative assessment of the pelvic and inguinal lymphatics to date. An overview of the methods that have been used in the past to record and communicate anatomic information provides a foundation for new approaches to translating these findings into clinical practice.

The origins of anatomical study can be traced back to ancient Egypt. The Ebers Papyrus of 1550 BC provides a written record of ancient Egyptian medicine that provides a description of the heart and its vessels\textsuperscript{204, 205}. This represents one of, if not the first, recorded symbolic representations of human anatomy. Following this period, the later works of Hippocrates (460-377 BC) and Galen (129-200/217 AD) document the medical knowledge within ancient Greek and Roman society in both symbolic and pictorial forms\textsuperscript{206, 207}. Both Hippocrates and Galen have had a profound and lasting
influence on Western medical thought. Roman law, during the time of Galen, forbade human dissection and anatomic investigations were primarily limited to the study of non-human primates, pigs and other mammals. Galen’s works would later be supplanted by those of Andreas Vesalius (December 31, 1514 - October 15, 1564). Vesalius, unlike Galen, faced no similar constraints and undertook careful and methodical observational studies of human anatomy, which resulted in the publication of *De humani corporis fabrica*\(^{208}\). Galenic anatomy, because of the limitations during that period, contained many inaccuracies. Through a fastidious focus on detail and iterative refinements, Vesalius was able to refute the errors in Galenic teachings and raise the standard for medical works with the creation of his anatomic atlas\(^{207}\).

This initial atlas and subsequent ones provided a means of communicating knowledge through pictorial representations as opposed to text alone. Accurate visual depictions of human anatomy can convey the classification of spatial objects, their structural networks (topological boundaries), the relevant part-of networks (component parts of objects of interest) and the spatial associations between these objects and adjacent related or unrelated objects\(^{209}\). Figure 3.1C depicts a three-dimensional rendering of the arterial and venous vasculature of interest in addition to the lymphatics and relevant musculoskeletal anatomy that readily conveys the relationships and topographic boundaries among these structures. The component parts are similarly appreciable in the vasculature and its bifurcations. Anatomy atlases can facilitate the generation of mental constructs through a representation of reality that acts to record and convey spatial and structural complexity \(^{210-215}\) and are fundamental cornerstones of understanding and teaching. Continued revision, the incorporation of new knowledge
and improvements in the manner of its representation help these atlases to be better utilized for research and clinical care delivery.

Paper, derived from the Greek for papyrus - the medium used by the ancient Egyptians, affords primarily a two-dimensional means to record anatomical information\(^{204, 205}\). Early three-dimensional anatomic representations were undertaken as evidenced by the wax models created under the supervision of Paolo Mascagni\(^{216-218}\). While of high anatomic fidelity, they were limited as a means of conveying the individual anatomic vagaries that are present within a population and were both labor and cost intensive. More recently, advances in computer based medical imaging and image analysis have provided a means of developing three-dimensional digital anatomic models. These virtual atlases have been generated for a variety of anatomic sites based on representative individuals, as well as for populations\(^{219-222}\).

Neuroanatomy and functional mapping have utilized atlases with defined coordinate systems using data derived from a carefully studied individual\(^{221}\). Relating features known for a “representative” individual to a particular subject of interest is undertaken via application of a defined set of proportionality rules using a common reference space. For anatomic structures that do not demonstrate significant inter-subject variability, and for which any variability that is present can be addressed through the application of proportionality rules, the use of one single average subject is appropriate. However, a single average subject poorly represents anatomical structures with inherent random variability. The USPIO studies described in this work as well as the work published by
Taylor et al. demonstrate sufficient random variability in pelvic lymphatic and vascular anatomy to argue against the use of a single individual or small sample\textsuperscript{169, 183}. In representing structures with a large degree of inter-subject variability, such as the pelvic and inguinal lymph nodes, a mathematical model that describes the spatial distributions and topographic relationships derived from a large population is more apt to provide a representative reflection of the structure of interest and facilitate differentiation of sub-populations\textsuperscript{222}. Such atlases have been both probabilistic and statistically based. Probabilistic atlases provide an “average” depiction of some feature of interest in reference to a standard space or co-ordinate system for a collection of individuals. Statistical atlases depict the variability within a population and facilitate the evaluation of factors that account for the anatomic variability within the population and allow the shape properties of anatomic structures and the spaces between them to be explored\textsuperscript{223}. The papers by Taylor et al., Shih et al. and Dinniwell et al. demonstrate to varying degrees the potential utility of such an approach\textsuperscript{169, 170, 183}. No one study however fully exploits the potential of either a probabilistic or a statistical lymphatic anatomic atlas.

From a broader perspective, there is also ongoing work to develop a standardized representation of anatomic ontology to create the Digital Anatomist Symbolic Knowledge Base\textsuperscript{215}. This work focused on the symbolic representations and spatial relationships of anatomic structures to produce an adaptable and integrated knowledge base of systemic and regional anatomy that can be queried at varying levels of physical scale\textsuperscript{215}. Together a model of anatomic variability and of complex relationships
expressed through a standardized ontology would facilitate knowledge management, the incorporation of future data and the provision of clinical care.

At present, no such model exists, with the topographic lymph node atlas developed by Qatarneh et al. representing the closest approximation to a whole body atlas\textsuperscript{224}. Unlike the model developed by Talairach, the model based on the Visible Human Male lacks any means of scaling the anatomic properties or features to other individuals, which curtails its utility\textsuperscript{225}. The transition from film to digital image processing in radiology has facilitated computer based quantitative image analysis. The introduction of the DICOM (Digital Imaging and Communications in Medicine) standard for storing digital imaging information allows large cross-sectional anatomic image series to be rendered and viewed in two- or three-dimensions for volumetric analysis. Two prior studies and those presented in Chapters 2 and 3 use the digital images from USPIO-enhanced MR imaging to derive lymph node clinical target volumes for radiotherapy treatment planning of pelvic malignancies\textsuperscript{169, 170, 183}.

The model described by Shih et al. is comprised of 18 prostate cancer patients (10 newly diagnosed and 8 with persistent or recurrent disease)\textsuperscript{170}. Within the group were 69 metastatic lymph nodes with a range of 1 to 21 (median 2 mean 3.8) identified per patient. Fourteen of the 69 lymph nodes were situated in a para-aortic distribution, and the remaining 55 were in the pelvis. The majority of the metastatic lymph nodes (40 of 69) were $\leq$ 1 cm in size. Using the 69 metastatic lymph nodes, a model was generated with the center point of each lymph node plotted in spatial relation to the adjacent anatomy. The center point was expanded for all nodal centers by a uniform 5 mm
margin to yield a sphere 1 cm in diameter, which was taken to be representative of the node. The distance from the surface of each representative nodal sphere to the surface the adjacent blood vessels was then assessed to determine that 94.5% of lymph nodes were encompassed with a 2.0 cm margin of expansion around sections of the common, external and internal iliac vessels. This study has several limitations. Only “metastatic” lymph nodes were included in the analysis and the topologic distribution of radiographically normal lymph nodes at risk of harboring occult micrometastatic disease was not addressed, thereby limiting this as a useful guide for defining target volumes for adjuvant radiotherapy. All lymph nodes were assumed to be adequately represented as 1 cm spheres, which appears to run counter to earlier works examining the short-axis dimensions of the retroperitoneal, pelvic and inguinal lymph nodes. Grubnic et al., using a sample of 12 patients who had undergone lymphangiographic, MRI and CT studies, determined the 95th percentile maximum sort axis dimension of pelvic lymph nodes to be: 4 mm for the common iliac and obturator, 5 mm for the external and internal iliac 5 mm and 6 mm for the hypogastric. Within the retroperitoneum, the 95th percentile maximum short axis dimension was 3 mm for the paracaval and inter-aortocaval nodes, 4 mm for the posterior caval nodes, and 5 mm for the low left para-aortic nodes. Normalizing all of the nodes to 1.0 cm for all nodal stations regardless of the underlying anatomy, could have resulted in additional adjacent normal tissue being unnecessarily encompassed in the vascular expansion used to form the nCTV. These potential problems have limited the clinical acceptance of this model.
A related study by Taylor et al. examined 20 patients, all of whom were newly diagnosed with endometrial or cervical carcinomas\textsuperscript{169}. None of the patients was reported to have pathological lymph node metastases. A total of 1216 nodal contours were generated, ranging from 30-101 (median 58) in individual patients. Of these, 627 were from the external iliac region, 303 from the obturator region, 144 from the internal iliac region and 135 from the common iliac region (under represented as only 13/20 patients were imaged with an adequate field of view). The nodal contours had a mean short axis of 3.6 mm with a standard deviation of 1.6 mm (1.1 to 12.1 mm). Only seven contours (0.6\%) were greater than 10 mm. Following identification and delineation of the lymph nodes, varying margins of expansion were applied to the adjacent vasculature and the percentage lymph node coverage was calculated. It was determined that a 7 mm margin encompassed 88\% of the adjacent lymph nodes. With the addition of a 10 mm presacral margin anterior to S1/S2 and an 18 mm margin along the lateral pelvic sidewall between the external iliac volume anteriorly and the internal iliac volume posteriorly, all demonstrable lymph node tissue could be encompassed. In a follow-up validation cohort using a series of 10 patients with 741 identifiable nodal contours, application of these margins resulted in inclusion of 737 of the contours. A 17 mm sidewall margin was found to be adequate rather than the 18 mm margin proposed originally. Despite limitations in the field of view and the absence of data concerning nodal frequency as opposed to contours, this study is useful for its assessment of the distribution of lymph nodes in relation to the adjacent vessels.

The model proposed by Taylor et al. represents an improvement over the one by Shih et al. in better reflecting the differences between the distinct vascular segments and the
importance of considering adjacent anatomy when delineation nodal target volumes\textsuperscript{169,170}. However, difficulties arise from the manner in which the lymph node data were reported, and this limits comparison with the existing published literature. Qaterneh, in an analysis of the high resolution National Institute of Health Visible Human Male data set, identified 6 internal iliac, 14 obturator, 1 promontorial, 3 sacral and 6 common iliac lymph nodes\textsuperscript{224}. Grubnic \textit{et al.} reported mean nodal numbers: of 11.9 (4-18) for the common iliac, 10.4 (1-16) for the external iliac, 4.8 (1-9) for the obturator, 5.1 (0-13) for the internal iliac and 1.8 (0-4) for the hypogastric\textsuperscript{227}. The long-axes of a significant number of these nodes extended across more than one axial plane. However, instead of lymph nodes, Taylor \textit{et al.} reported their data using nodal contours and nodal frequency for each site was not specified. Similarly, the short axis dimension was not reported in a manner consistent with the published literature\textsuperscript{230-232}. By convention, there can only be one short-axis dimension per lymph node. However, in reporting nodal contours, a median short axis distance of 3.6 mm with a standard deviation of 1.6 and a range of 1.1 to 12.1 was described\textsuperscript{169}. Lymph nodes and nodal contours were used interchangeably in this study, which might have introduced a left skew to the distribution by repeatedly sampling multiple “short-axes” for a given lymph node. They cannot then be meaningfully compared to other reported series. Unlike Shih \textit{et al.}, the volumes proposed by Taylor \textit{et al.} did incorporate the influence of adjacent musculoskeletal and visceral anatomy with modifications that effect a transition from an isotropic to an anisotropic distribution\textsuperscript{169,170}. This helps to ensure adequacy of lymph node coverage while facilitating normal tissue avoidance.
Both of these studies have been incorporated into expert consensus nodal volume definitions. A Radiation Therapy Oncology Group Genitourinary (RTOG GU) expert panel was assembled to review material from lymphographic studies and the results of extended surgical and sentinel lymph node dissections, in addition to the studies by Shih and Taylor\textsuperscript{233, 234}. It was concluded that the patterns of prostatic lymphatic drainage were primarily to the internal iliac, external iliac and sacral lymphatic nodal basins. The recommendation was for a 7 mm uniform vascular expansion with pelvic sidewall strips of unspecified width and a 10 mm strip anterior to S1-S3 to encompass the presacral space. The RTOG Gynecologic Oncology (GYN) expert panel reviewed source material from anatomical and surgical atlases, surgical texts, imaging references, as well as IMRT and patient experiences to derive their target volumes\textsuperscript{235}. Similar to the GU guidelines, they recommended a 7 mm vascular expansion with a 15 mm strip anterior to the sacrum. A further margin to unite the external and internal iliac contours along the lateral pelvic sidewalls was recommended but not quantified. The RTOG Gastrointestinal (GI) group, having identified an educational need for nodal clinical target volume delineation, formed a consensus panel and each member was asked to delineate the clinical target volume for a representative case. Using these target volumes, 95\% confidence level consensus contours were developed\textsuperscript{236}. From this analysis, it was concluded that a 7 to 8 mm margin of expansion around iliac vessels (\(\geq 10\) mm anterolaterally) and a 10 mm strip anterior to the sacrum should be used. No specific margin of expansion was recommended for the femoral vessels. The inguinal lymph node target volume was to be delineated as a compartment and not based on vascular expansion. Table 4.1 summarizes the recommendation from prior studies and the consensus definitions.
Table 4.1: Comparison of recommendations for nodal CTV delineation guidelines

<table>
<thead>
<tr>
<th></th>
<th>Shih\textsuperscript{170}</th>
<th>Taylor*\textsuperscript{169}</th>
<th>RTOG GU</th>
<th>RTOG GYN</th>
<th>RTOG GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Iliac AV</td>
<td>20 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td></td>
</tr>
<tr>
<td>External Iliac AV</td>
<td>20 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7-8 mm</td>
</tr>
<tr>
<td></td>
<td>(18 mm Distal Ant/Lat)</td>
<td></td>
<td>(10 mm Ant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal Iliac AV</td>
<td>20 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7-8 mm</td>
</tr>
<tr>
<td>Obturator (Lateral pelvic sidewall)</td>
<td>18 mm</td>
<td></td>
<td>Not specified</td>
<td>7 mm</td>
<td>Not specified</td>
</tr>
<tr>
<td>Presacral space</td>
<td>10 mm</td>
<td>10 mm</td>
<td>15 mm</td>
<td>≥10 mm</td>
<td></td>
</tr>
</tbody>
</table>

*7 mm expansion encompassed 88% of lymph node contours so modification recommended
In the work presented in Chapters 2 and 3, unlike Shih et al., all gross nodes were censored and no attempt was made to generate representative nodes in their place, since the main objective was to develop clinically relevant target volumes for adjuvant radiotherapy\textsuperscript{183}. Given the small number of such nodes, it is not likely that this had any meaningful impact on the results. As with Taylor et al., the volumes were modified to reflect differences in each nodal vascular segment and the adjacent anatomy\textsuperscript{169, 170}. In contradistinction to Taylor et al., who determined the isotropic vascular expansion required to include a set amount of the adjacent lymph node tissue and then modified it to capture the remainder, the method outlined in Chapters 2 and 3 aims to capture all lymph nodes and then modify the volume obtained to exclude adjacent non-lymphatic anatomy. Despite the apparent difference in the proposed margins of expansion between Taylor’s study and ours, the derived nodal CTV’s are actually quite similar. The manual contour modifications that are implicit in the Taylor et al. CTV definition, in effect, further expand their 7 mm vessel margins so that the final volumes are relatively coincident\textsuperscript{169, 170}. Our approach may be less time consuming and less prone to error because less revision is required for individual cases.

The greatest limitation of our work as well as previous studies is the inability of MR imaging with lymphotrophic USPIO’s like ferumoxtran-10 to identify small lymph nodes <3 mm in size, and microscopic lymph node metastases. The nodes comprising the presacral and internal iliac groups are likely underrepresented in all of these studies by virtue of their size. Taylor et al. reported the frequency of lymph node contours and a summation of contours through successive axial images would be required to determine the number of lymph nodes in each nodal group\textsuperscript{169}. Within their 20 patient series, a
mean of 0.4 (0-2) and 7.2 (1-22) lymph node contours were identified for the presacral and internal iliac lymph node groups respectively. In comparison, a median 2 (0-6) and 5 (0-15) presacral/subaortic and internal iliac lymph nodes were identified in our 55 patient series. Recognizing that the presacral lymph node group in our series included subaortic lymph nodes and that nodes within each group may lie across more than one adjacent axial plane, there would appear to be an equivalent or greater number of lymph nodes identified in our series with a larger sample size.

Our model for nodal CTV definition was derived from the spatial distribution of visible lymph nodes, on the assumption that these would be representative of all nodes at risk of harboring disease. It is possible that there are small lymph nodes at greater distances from the major vessels that were not visualized using this approach. The smallest short axis dimension of the lymph nodes delineated in the high resolution Visible Human Male dataset was 2 mm. While this is smaller than the slice thickness (3 mm) used in this study and raises concerns about identifying these lymph nodes using MRI, the close relationship of these lymph nodes to the adjacent vessels would result in them being encompassed in a nodal CTV generated using a margin of expansion derived from larger visible nodes. The adjacent lymph nodes that are of a larger size than the slice thickness used, and are apparent on the MRI images, can help to define the outer boundaries for an anatomic envelope to encompass the nodal basin within which smaller (<3 mm) lymph nodes may reside. However, these are likely to represent a small proportion of the total nodal volume in each anatomic region and would not substantially alter the proposed margins of expansion, which were defined to encompass 90% of the lymphatic tissue in 90% of patients. Ferumoxtran-10, while
lowering the MR threshold for the detection of subclinical disease, does not enhance the sensitivity enough to reliably detect microscopic metastases. This currently limits the utility of this approach for defining patient-specific radiotherapy volumes that target only regions of known tumor.

All of the inherent limitations in our work and other previous studies of pelvic lymph node target volume definition (adequacy of anatomic coverage, knowledge of patterns of spread, patient specific nCTV delineation) could be mitigated in whole or in part with the use of imaging techniques and contrast agents that are more sensitive and specific for the detection of small lymph nodes and microscopic lymph node metastases. Unfortunately, there is no lymph node contrast agent currently available for clinical use. In the 55 patients analyzed to determine pelvic nodal target volumes (Chapter 2) and the 30 patients that formed the basis for the inguinal target volume definition (Chapter 3), it has been demonstrated that a population based class solution can be derived for isotropic margins of vascular expansion. While the model would benefit from a larger sample size to ensure sufficient power for clinical use, it is instructive in its demonstration of the potential of isotropic margins. These margins are capable of encompassing the lymph nodes of interest while reducing the volume of normal tissue included in the nCTV. A reduction in the volume of normal tissue within the high dose nCTV may help lead to a reduction in the acute and late toxicities from pelvic radiotherapy.

A further incremental improvement might be realized with the use of non-uniform margins of expansion or the addition of further vascular segments upon which margins
of expansion may be applied. Within each vascular segment, characteristic distributions of the lymph nodes are apparent, as illustrated in Figure 4.1. The common iliac lymph nodes appear to be closely related to the posterior aspect of the adjacent vessels while the external and internal iliac lymph nodes are situated more circumferentially and at greater distances from the vessels. These differences primarily arise because of the relationship of the vessels and nodes to the adjacent musculoskeletal anatomy, including bone and muscle. While anisotropic expansion is a potential refinement on isotropic margins, still further improvement could be obtained with continuously variable patient-specific margins around vessels based on more sensitive and specific nodal imaging techniques.

The guidelines derived for pelvic and inguinal nCTV's in Chapters 2 and 3 are based upon the paired relationship between regional lymph nodes and adjacent vascular segments. Within the femoral triangle, lie the femoral artery and vein in addition to the great saphenous, superficial circumflex iliac, superficial epigastric, external pudendal as well as the lateral and medial accessory saphenous veins (Figure 1.8 and 1.9). In exploring the spatial distribution between the lymph nodes and vascular segments, the impact upon the histogram plot of the addition of vascular segments to the femoral artery and vein is readily apparent (Figure 2.3). Using the femoral artery and vein alone, a larger margin of expansion is required to encompass the lymph nodes and the centre of balance appears to be about 15 mm. With the addition of new vascular segments, the balance shifts left with an associated reduction in the margin of expansion required to encompass an equivalent volume of nodal tissue. In Figure 2.3, the skewness of the histogram shifts left with the incorporation of the great saphenous and superficial
circumflex iliac veins along with a slight reduction in the range. Those lymph nodes (lateral superficial and inferior superficial) which had been encompassed by an over expansion of the femoral artery and vein are now paired with their corresponding artery or vein. Were it technically possible to identify, delineate and add the remaining vascular segments, the histogram plot would assume a normal distribution with a balance less than 10 mm and a narrow range. In an effort to ensure an accurate and efficient means of nCTV delineation, those vessels which are difficult to appreciate in the absence of vascular contrast or additional imaging modalities have been omitted. Were there a clinically practical means of defining further vascular segments such as the branches arising from the internal iliac artery and the veins and arteries within femoral triangle, a re-analysis of the data presented in Chapters 2 and 3 could be undertaken to generate a population based statistical library of vascular segment expansions that could be applied to individual named vascular arterial or venous branches. The long intravascular circulatory time of the USPIO does enable MR arteriograms and venograms to be obtained from which those smaller vascular branches could be reliably segmented. This would yield an iterative refinement in the data presented in Chapters 2 and 3 in addition to a reduction in the individual vascular margins of expansion.

A similar effect is noted in the histogram shown in Figure 2.3. In delineating only the proximal internal iliac vessels cephalad to their origin and extending the object delineations along the course of the superior gluteal vessels, the remaining branches of the internal iliacs are omitted from the analysis (Figure 1.8 and 1.9). This is also the case for the middle sacral artery. Through the delineation of anatomic land markers for
Figure 4.1: Axial MR images for: (A) the common iliac vessels and common iliac lymph node (red arrow); (C) the external iliac vessels and external iliac lymph node (white arrow) and (E) the internal iliac vessels and an internal iliac lymph node (blue arrow) and the corresponding scatter plot distributions of the lymph node volume elements in relation to the surface of these vascular segments. Note: the red dot (0,0) denotes the point defining the vessel surface.
the lateral pelvic sidewall and the distal lumbar and anterior sacral surface, those nodes which would require an over expansion of the common, external and internal iliac vessels to assure their inclusion (data not shown). With the use of the land markers, their histogram plots remain with a narrower range and a balance more reflective of the underlying anatomy. Were those vessels that had been omitted to be delineated and included in the analysis, the anatomic land markers for the subaortic/sacral and lateral pelvic sidewall would confer no additional gain and could be eliminated..
Chapter 5. Conclusions and Future Directions

In conclusion, isotropic margins can be derived for the pelvic and inguinal vasculature. The application of these margins in the context of adjuvant IMRT for pelvic malignancies should enable consistency in lymph node clinical target volume delineation while facilitating normal tissue avoidance. Derivation of anisotropic margins of expansion may enable additional sparing of normal tissues while providing similar lymph node coverage to isotropic margins. Analysis of a larger sample size and validation would help to better power this model and assess its utility for clinical use.

Studies of IMRT in the treatment of pelvic malignancies have demonstrated reduced volumes of the small bowel, rectum, bladder and bone marrow in the high dose regions, with a resultant decrease in acute and chronic gastrointestinal toxicities\textsuperscript{132, 157, 158, 237-240}. Application of the modified isotropic or the anisotropic margins in a prospective clinical study with IMRT would help to determine any further reduction in toxicities related to the reduced volume of normal tissue irradiated using these nCTV margins.

At present, the limitations to patient specific USPIO imaging include: the absence of a clinically available agent, cost of the MRI and radiology reporting time (mean time to report one image series by an expert observer is 80 minutes with pre- and post-contrast imaging required), and the learning curve of the reporting radiologist\textsuperscript{174}. USPIO currently is not available in North America outside of a clinical trial. The models presented here as well as those reported by others represent means of using a population based model for the radiotherapy treatment planning of an individual patient\textsuperscript{169, 170, 183}. These nascent
anatomic models parallel the efforts that have recently been undertaken in other domains to develop an animated four-dimensional model of the human body using morphologic, functional and molecular information within a unified representation\textsuperscript{220}. The core elements of this model include: a 3-dimensional digital atlas of the human body, ontology-based metadata support and biomedical datasets\textsuperscript{220}. This will allow spatio-temporal modeling of complex disease processes and is sufficiently evolved to demonstrate changes over time reflecting progression of underlying pathology. In the context of the body of work discussed here, a depiction of the spatial topology of the lymph node distributions within the pelvis and groin as well as the pattern of local, regional and distant metastatic spread both within and external to the lymphatics could be incorporated into such a model. From a lymphatic atlas, composite organ systems could then be added and integrated into a unified and dynamic digital construct. Into this form, medical images and spatio-temporal data could be incorporated and manipulated in four-dimensions over a range of scales from the subcellular to the that of the entire organism.

A standard three-dimensional anatomic form affords a framework to which additional morphologic information can be added. For example, lymphatic vessels cannot be adequately imaged using currently available cross-sectional imaging approaches. However, with the use of microsurgical techniques and computed tomographic lymphangiography, the relevant efferent and afferent lymphatic vessels can be detected\textsuperscript{241}. This new information could be incorporated into a standardized three-dimensional anatomic model to refine currently available information about the micro-regional distribution of lymphatic vessels and lymph nodes.
Image data with USPIO is limited by MRI resolution. Additional complementary pulse sequences, such as diffusion-weighted MRI (DW-MRI), may enhance the diagnostic accuracy. DW-MRI is a non-invasive imaging technique that examines the structure of a biologic tissue and facilitates the assessment and measurement of tissue microstructure (Figure 5.1). The random thermal motion of water molecules, Brownian motion, results in tissue dependent signal attenuation. This allows tissues to be evaluated on the basis of differences in water-proton mobility within and between anatomic structures. DW-MRI has been investigated for a variety of pathologic conditions such as acute stroke, multiple sclerosis, abscesses and tumors\textsuperscript{242-247}. More recently, it has been used to serially monitor solid tumor response to chemotherapy over time and identify nodal metastases\textsuperscript{248, 249}. Changes in the mobility of water molecules in a tissue afford a non-invasive means of further characterizing a given tissue.
Figure 5.1: (Left) T2-w Fast Spin Echo MR image of the pelvis at the level of the acetabulum with (\) denoting a left lateral superficial inguinal lymph node. (Right) DWI MR image at the level of the acetabulum with (\) denoting a left lateral superficial inguinal lymph node.
A recent report by Thoeny et al. examined the potential of DW-MRI to facilitate reader reporting of USPIO-enhanced MR imaging studies of normal sized lymph nodes in 20 patients with bladder or prostate cancer\textsuperscript{174}. The authors evaluated the potential diagnostic synergism between USPIO and DW-MRI using a 3-T MRI with pre- and post-USPIO imaging series. At the completion of imaging, all patients underwent an extended pelvic lymphadenectomy and resection of the primary tumor. The addition of DW-MRI failed to improve the overall diagnostic accuracy for lymph node metastases. However, DW-MRI did appear to facilitate nodal identification, as the reporting time for the combined arm was less than that for the USPIO arm (mean of 13 minutes vs 80 minutes respectively, \( p<0.0001 \)). Eight percent (2 of 26) metastatic lymph nodes were missed by imaging. These nodes had dimensions of 1.0 x 0.2 mm and 0.7 x 0.4 mm. This highlights an important limitation of commonly-used MR technology and pulse sequences in relation to the detection of micrometastatic disease.

The diagnostic accuracy of CT or MR for the detection of lymph node metastases may be improved by integrating these anatomic imaging modalities with functional and molecular PET or SPECT imaging. This creates the potential for synchronized acquisition of morphologic, functional and molecular information\textsuperscript{250}. While historically of limited resolution, enhancements such as pinhole SPECT have enabled the detection of discrete nodal metastases in sites such as the axilla\textsuperscript{251}. As further improvements are made in camera design with greater intrinsic spatial resolution, the potential for additional discrete nodal metastasis detection will increase.
Additional improvement in nodal detection can be obtained by increasing the inherent contrast between the different tissues of interest (e.g. lymph node versus adjacent non-lymphatic anatomy) with the administration of a contrast agent. Radiographic lymphography has recently re-emerged for axillary lymphatic mapping with the interstitial administration of commercially-available water-soluble CT contrast media into the breast\textsuperscript{89, 241, 252-254}. Unlike the current sentinel lymph node technique, 3D-CT lymphography provides a permanent record of the route and pattern of lymphatic drainage from the peri-tumoral injection site within the breast through to the first, second and third order draining lymphatics. Optical fluorophores, quantum dots, have also been used to identify the sentinel lymph nodes in animal models. They consist of nanometer sized (5 to 20 nm) cadmium-selenium or cadmium-tellurium semi-conductor crystals\textsuperscript{255}. Quantum dots emit energy over a narrow wavelength that is determined by their size and shape. Therefore, using quantum dots with different characteristics allows an assessment of routes of transport in tissue. As with 3D-CT breast lymphography, the use of quantum dots to image lymph node drainage basins is currently limited to interstitial peri-tumoral administration as they are not taken up by the lymphatics following intravenous infusion\textsuperscript{255, 256}. Other limitations include the potential for toxicity from the semi-conductor core elements \textsuperscript{[261]} and the production of free radicals from the optical excitation energy leading to DNA damage and cell death\textsuperscript{257}. While the unique physical properties of quantum dots give them utility in the study of animal model systems, the current toxicities and lack of lymphotrophism are impediments to clinical translation.
Adenoviruses are known to be lymphotrophic\textsuperscript{258}. This may be a consequence of their size, negative charge or some additional biological feature. Recombinant adenoviruses have been created to act as gene delivery vectors. Imaging reporter genes have been successfully inserted into prostate-specific adenoviral vectors and detected following administration of the virus in an animal model\textsuperscript{259}. Unfortunately, in spite of the known lymphotrophism, only a small fraction (0.1\%) of the adenoviral dose is detectable in lymph nodes following interstitial administration. \textsuperscript{258} This along with the potential for incomplete gene transfer may limit its sensitivity and subsequent clinical utility.

The overall quality of all of the different imaging modalities is improving and with this progress, the potential for individual lymph node identification and segmentation will become more accessible in the clinic. Using high-resolution isotropic voxels and segmentation algorithms, one can volumetrically identify the lymph nodes of interest within a given anatomic site. Currently available commercial software allows automated segmentation and analysis of lymph nodes following manual identification of seed points to guide this process\textsuperscript{260}. The nodes may then be analyzed in two- and or three-dimensional visualizations using accepted means, such as those specified by the response evaluation criteria in solid tumors\textsuperscript{231, 261}. This potential has also been explored by others\textsuperscript{262, 263} and can be improved upon by subdividing regions, as was presented in Chapters 2 and 3 for inguinal and pelvic lymph nodes. Region specific delimiters for the head and neck, axilla, thorax, abdomen, pelvis and groin would enable further refinement of these methods.
In summary, this study has highlighted the importance of high resolution lymph node imaging with an appropriate contrast agent in the current era of high precision radiotherapy. USPIO offer several advantages for the detection of lymph nodes and lymph node metastases that can aid in radiotherapy target volume definition. Contrast agents are not currently available for routine clinical use. However, their demonstrated utility will help to ensure that they (or other newly emerging nodal imaging agents) will eventually find a place in the diagnosis and treatment of cancer, leading to greater individualization of care. In the absence of widely available nodal contrast agents for patient-specific treatment planning, the use of class solution nodal clinical target volumes based on contrast-enhanced high resolution nodal imaging as presented here will form the foundation for current patient care and the development of innovative treatment strategies in the future.
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