



Sentinel Node Methods in Penile Cancer – a Historical Perspective on Development of Modern Concepts

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Malignant penile tumors are of squamous cell origin in more than 95% of cases and the occurrence of a distant metastasis without prior inguinal lymph node metastatic deposits is very rare. This makes inguinal lymph node staging very reliable and of great prognostic significance since undiscovered and untreated inguinal metastases may lead to a fatal clinical course. In lack of a sufficiently accurate noninvasive lymph node staging modality, penile cancer relies on surgical lymph node removal for regional staging. In this respect sentinel node biopsy offers a favourable minimally invasive alternative to prophylactic inguinal lymph node dissection which is associated with significant surgery-related morbidity.

Today sentinel node biopsy is widely used in surgical oncology within high volume cancers such as breast cancer and melanoma. In rare cancers sentinel node biopsy is also emerging as a minimal invasive staging tool in patients with no obvious lymph node involvement. At several specialized units across Europe sentinel node biopsy has been practiced by dedicated specialist within vulva and penile cancer for more than two decades. In fact, the rare disease penile cancer was a model entity for development of the original sentinel node concept as early as the 1970'es due to work by the Paraguayan penile cancer pioneer, Cabañas, the sentinel node concept was subsequently successfully adapted in breast cancer and melanoma. This turned out mutually beneficial since the sequential development of sentinel node biopsy in penile cancer in the 1990s eventually adopted new insights and added conceptual details from the experiences harvested in the broader clinical application possible in these high-volume diseases.

The prerequisite to conceptualising the sentinel node approach was the gradual anatomical and functional understanding of the lymphatic system which in western medicine rooted in ancient Greece and gradually increased in details and comprehension with significant contributions from many great notabilities during the last centuries including Hippocrates, Galen, Fallopio, Malpighi, Virchow, Starling, Cabañas, Hodgkin and Horenblas.

Sentinel node biopsy in penile cancer is a complex multimodality procedure involving inguinal ultrasonography by radiologists, precise tracer-injection and interpretation of nuclear images by nuclear medicine physicians, radio-tracer- and dye guided open surgical biopsies by urologists and thorough step-sectioning, immunostaining and accurate lymph node specimen analysis by pathologists. This team effort requires well-tested protocols, experience and good collaboration and in rare diseases this calls for centralization of service.

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Penile Cancer

Incidence and Incidence Trends

With an age-standardized incidence rate at 0.84 cases per 100,000 person-years worldwide penile cancer is a rare entity.¹ Most publications on penile cancer report a stable or only slightly varying incidence depending on the database studied

and the methods applied.²⁻⁹ Publications from the United States and Finland reported a slightly decreasing incidence.^{2,6,10} On the other hand, a slightly increasing incidence was reported from British, Dutch, and Danish registries.^{3,7,9} Another Danish study reported a stable incidence, and the authors argued that increasing incidences in the studies by Baldur-Felskov (DK) and Arya (UK) rely on questionable evidence and methodology.^{4,5,8,11} Current penile cancer incidence trends might be varying across the globe and controversial in certain geographic regions, however, the global demographic development points to a rising incidence in the future.^{1,12} Men can be affected by penile cancer as early as in their twenties, but the age-related incidence peak is around 60 years of age, and the incidence increases with increasing age.^{1,2} World population ageing is accelerating, and projections indicate that by 2030, persons above 60 years of age are expected to account for more than 25% of the populations in Europe and in Northern America, 20% in Oceania, 17% in Asia and in Latin America and the Caribbean, and 6% in Africa.¹² This demographic development alone is expected to entail an increase in the total incidence of penile cancer especially in Europe and Northern America.^{1,12,13}

Risk Factors

No association between penile cancer and sexually transmitted bacteria has been established, whereas human papilloma virus (HPV) is considered a risk factor along with smoking and lichen sclerosus et atrophicus.¹⁴⁻²³

Prognosis

Prognosis in penile cancer is dependent on tumor node and metastasis (TNM) parameters and especially the decisive significance of nodal stage cannot be exaggerated.²⁴⁻²⁷

The Lymph Node Dilemma in Clinically Node Negative Patients (cN0)

At diagnosis, around 75%-80% of penile cancer patients are clinically node negative (cN0).²⁵ When examined pathologically, between 20% and 25% of cN0 patients harbor inguinal lymph node metastases.²⁵ This is a true clinical dilemma since prophylactic inguinal lymph node dissection in all cN0 patients would lead to over-treatment of 75%-80% with possible complications from this treatment.^{28,29} On the other side, a wait and see approach without inguinal lymph node evaluation in cN0 patients leads to excess mortality from penile cancer.²⁴⁻²⁶

With insufficient spatial resolution of current imaging modalities in terms of demonstration of micro-metastatic disease, the need for a minimally invasive nodal staging modality is obvious.³⁰⁻³² This need is met by sentinel node biopsy, a modality which was gradually developed during the last fifty years.³³ During the development of the sentinel node concept, important pioneering findings were made within penile cancer.³³⁻³⁶

The development of the sentinel node biopsy rests on very important conceptual cornerstones of the lymphatic system which has a long and interesting history dating back to the father of modern medicine, Hippocrates.^{33,37-40}

The Discovery of the Lymphatic System – Ancient Greece

Hippocrates (ca 460-370 BC) presented a course of disease consistent with cancer metastasising to lymph nodes and is believed to have fathered the term, *Karsinos*, cancer.^{37,38} Hippocrates noted further findings of fibrous structures between blood vessels and nerves containing what he termed “white blood.”³⁷ The Alexandrian anatomist Herophilus (335-280 BC) introduced vessels terminating in ovoid glands in the mesentery and his Alexandrian student and colleague Herasistratus (304-250 BC) also mentioned the milky contents of these vessels, which they wrongly believed were blood vessels.^{37,38} Herophilus and Herasistratus collected their knowledge from human dissections which were practised in Alexandria in their time.³⁷

On the other hand, the later Greek physician Galen of Pergamon (129-199 AD) worked under the constraints of Roman law forbidding dissection of humans. Thus, the important anatomical contributions of Galen rest on a series of animal dissections.³⁷ Galen described the mesenteric lymph nodes and abdominal lacteal vessels containing chyle.³⁷ The Byzantine Greek physician Paul of Aegina (607-690 AD) collected available contemporary knowledge of anatomy, medical and surgical treatment as well as midwifery in an important encyclopaedia, the *Medical Compendium in Seven Books* from which knowledge of tonsil infections involving neighbouring lymph nodes in the cervical region were echoing well into the Middle Ages.^{37,41}

The Description of the Lymphatic System 1500-1850

From the early 17th century and onwards an increasingly detailed description of lymphatic vessels arose. Gabriello Fallopio (1523-1562) leaving his name for posterity by describing the fallopian tubes (*tubae uterinae*) discovered during his time as the chair of the anatomy and surgery department in Padua, Italy, also encountered mesenteric “veins” which upon opening spilled “yellow matter.”³⁷

The Italian anatomist Bartholomeus Eustachius (1500-1574), famous for discovering the eustachian tube, documented the milky coloured duct “*vena alba thoracis*” (*ductus thoracicus*) in 1552 during a horse dissection.^{37,42,43} Seventy years later, in 1622, followed the discovery of lymphatic vessels in richly fed dogs by the Italian anatomist Gasparo Asellius (1581-1626).⁴⁴

In 1651 the Danish anatomist Thomas Bartholin the older (1616-1680) was the first to describe and name the human lymphatic vessels in his work *Vasa Lymphatica* published in 1653.⁴⁵ Also in 1651, a young French physician fresh out of medical school, Jean Pecquet (1624-1674), published the anatomic relations of the thoracic duct and its relations to

cisterna chyli and the abdominal lymph vessels.³⁷ A number of contemporary anatomists such as Olaus Rudbeck (1630-1702) from Uppsala, Sweden and George Joyliffe (1621-1658) from Oxford, England, confirmed the findings of Pecquet and Bartholin during the same period and it remains controversial and nationally coloured to this day who is credited for the discovery.^{46,47}

On the microanatomic level Marcello Malpighi (1628-1689) was the first to observe and document capillaries linking the pulmonary arterial circulation and the venous system. Malpighi also described nodal structures lying along the course of the lymphatics solitary and in groups.³⁷ The London brothers William Hunter (1718-1783) and John Hunter (1728-1793) worked with William Cruikshank (1745-1800) on several series of dissections of many different animals and using mercury injection they were able to demonstrate the progression of fluids through lymph vessels and produce illustrations of lymph vessel anatomy.^{37,48,49} Cruikshank published *The anatomy of the absorbing vessels* in 1786.^{48,49} More than a century before that, lymph node involvement in malignant disease had been described by surgeons. Among others the French surgeon Ambroise Paré (1510-1590) described lymph node involvement in malignancy, but not until 1844, the first lymphadenectomy was reported by the American surgeon Joseph Pancoast (1805-1882).³⁹

Clinical Utilization of Increasing Knowledge of the Lymphatic System 1850-1950

Already in the early 17th century the surgeon Marco Aurelio Severino (1580-1656) from Naples, Italy performed mastectomies with axillary dissection.³⁷ The French Jean Louis Petit (1674-1760) demonstrated the spread of mammary cancer to the axillary lymph nodes.³⁷ Spread of cancer along the lymph system was further described by the French surgeon Henri Francois Le Dran (1685-1770) but was not embraced by the medical community and incorporated systematically in surgical practice until a century later.^{37,38,39}

In 1832 British pathologist Thomas Hodgkin (1798-1866) published an article on malignant enlargement and deformation of lymphoid tissue.^{37,50} A quarter of a century later in 1856 the German pathologist Robert Virchow (1821-1902) published the first in a series of studies focusing on the roles of lymph nodes and the lymph node involvement in cancer. Virchow never made use of the term sentinel node, but the sum of ideas and hypotheses presented in his works sketched the primary backbone of the mindset and techniques of the sentinel node concept employed a century later.^{40,50,51}

The British Physiologist Ernest Henry Starling (1866-1927) is best known for his analysis of the heart's work as a pump, but also within the history of the understanding of the lymphatic system Starling contributed significantly to the understanding of functionality and mechanisms. Starling proposed that lymph formation is brought about because water is forced out of the capillaries through the pores in the capillary wall by hydrostatic pressure and retained by the osmotic pressure of plasma proteins. The two opposing

forces approximately balance but leads to a continuous secretion of fluids into the tissues. The fluid later returns to the circulation via a network of draining lymphatic vessels.⁵²

In 1895, the influential and controversial American surgeon William Halsted (1852-1922) published a systematic approach to axillary lymph node dissection and promoted the idea that cancer spreads to the lymph nodes first and from there to visceral organs as a predictable principle.^{38,39}

American Pathologist Ernest Tyzzer (1875-1965) opposed the validity of this principle and nuanced the matter in 1913, with the presentation of several cancer patients with distant metastases, but without regional lymph node involvement. Nevertheless, after Halsted's publication, lymph node removal became more common in the management of solid tumors.^{38,39}

Our Modern Understanding of the Lymphatic System Arise 1950-2021

Our modern view of metastatic dissemination of cancer with sequential or simultaneous escape of malignant cells into the lymphatic system and systemic circulation was formulated by American Surgeon Bernard Fisher (1918-2019) in 1967.^{53,54} Fischer's visionary model has been confirmed by the achievements of current molecular methods. Recently, we have increased our understanding of circulating tumor material, required characteristics of target tissues for metastases to settle and start to grow and in some cancers, we start to utilize this knowledge in treatment allocation and follow-up regimen.⁵⁵⁻⁵⁷

Sentinel Node Biopsy – Ramon Cabañas, a True Pioneer

Already two years before Fischer's visionary 1967 publication defined our modern understanding of the dissemination of cancers, a Paraguayan pioneer, Ramon Cabañas, had started to collect data for a very important study in the story of development of the sentinel node concept.

Some cancers have a predilection for a particular route of dissemination, and penile cancer only very rarely presents distant metastasis without prior dissemination via the lymphatic vessels to the inguinal regions. Because of this characteristic, penile cancer was an ideal model disease for the development of the sentinel lymph node concept.³

In 1977, the Paraguayan urologist Ramon Cabañas published a 100-patient lymphangiogram study demonstrating the proposed primary draining lymph nodes in penile cancer around the superficial epigastric vein. The study period was 1965-1973 and for the description of these primary draining lymph nodes Cabañas used the term "sentinel lymph node" suggested by the American surgeon Ernest Gould in 1960 in a small case series on eight parotid cancer patients.^{34,58}

Cabañas made an excisional lymph node biopsy of the presumed primary penile draining inguinal lymph node and termed the procedure 'sentinel node biopsy'. The procedure was undertaken in 46 patients with penile cancer and Cabañas found metastatic deposits at histological examination of

lymph nodes in fifteen patients, in whom he carried on performing a complementary inguinal lymph node dissection with removal of remaining inguinal lymph nodes and found additional metastatic nodes in only three (20%) patients.³⁴

In the 31 patients with negative sentinel node biopsy Cabañas did not perform further inguinal surgery and these 31 patients did not present further inguinal metastases during follow-up and had a 5-year survival at 90% compared to a 5-year survival at 70% for patients with positive sentinel lymph nodes and 50% in patients with additional metastatic nodes in the complementary inguinal lymph node dissection.

These significant results backed the idea of a stepwise and serial lymphatic spread of metastatic deposits from the primary tumor to a primary drainage site – the sentinel node(s).

The impressive findings by Cabañas relied solely on extensive experience with steadfast production and interpretation of technically challenging lymphangiograms and individual patient lymph node mapping by the means of surgical anatomy landmarks.³⁴ The pioneering Paraguayan surgeon did not use blue dye, radiotracer, scintigram or gamma probe, which were later technical additions to the sentinel node diagnostic armamentarium.⁴⁰

Few contemporaries of Cabañas' 1977 landmark publication had his experience with lymphangiograms and the method was difficult to reproduce. The findings were discredited due to the concern of overlooking metastases and have false negative results. It turned out that some of the scepticism was justified since casuistic publications and smaller case series published on false negative sentinel node biopsies.^{40,59-63} The anatomical comprehension of the sentinel node proposed by Cabañas did not take into account individual variation of lymphatic routing. For more than a decade the ideas of the sentinel node biopsy were not further developed.³⁸

Sentinel Node Biopsy in Melanoma and Breast Cancer, Morton, Krag and Alex

In 1992, the sentinel node concept was resumed and renewed in other disease entities than penile cancer.

In the field of melanoma, a surgical oncology landmark publication by Morton (1934-2014) and coworkers used blue dye to visualize lymph vessels and nodes.^{38,40,64,65}

During the following years the sentinel lymph node concept was widely adapted within melanoma and within the field of breast cancer. The benefit of adding radiotracer and per-operative gamma probe to the use of blue dye was published by Krag, Alex and coworkers in 1993.^{40,66,67} The advantages of visual and acoustic guidance towards the primary draining node improved the surgical technique and as opposed to the demanding injection technique of classical lymphangiograms where you must inject into small hard-to-localize vessels, the injection of dye and tracer more diffusely into the area of interest was less demanding and easier to reproduce. The sentinel lymph node technique had been improved and made more widely applicable. The advantage of developing the technique in diseases with a higher incidence than penile cancer also meant that development was more rapid.⁴⁰

For several years, sentinel node biopsy rather than full axillary lymph node dissection has been implemented as the standard of care for surgical staging in early breast cancer, unless axillary lymph node involvement has been proven by ultrasound-guided biopsy. Compared to full axillary lymph node dissection, sentinel node biopsy is associated with less surgical complications such as shoulder stiffness and arm swelling and allows for an accelerated trajectory and shorter length of stay. Within breast cancer, sentinel node biopsy recommendations rely on randomised trials.⁶⁸

Within the field of melanoma, sentinel node biopsy recommendations are based on nonrandomised studies.⁶⁹ For melanomas with a tumor thickness of >1 mm and/or ulceration sentinel node biopsy is currently recommended for precise staging.

Melanoma as well as breast cancer guidelines emphasise that sentinel node biopsy should be carried out by skilled teams in experienced centers.^{68,69}

Sentinel Node Biopsy in Penile Cancer Today

For two decades after the 1977 landmark publication by Cabañas on the early concept of modern sentinel node biopsy in penile cancer, not much was published on the subject.

The development within the fields of melanoma and breast cancer re-ignited attention on the idea. The next penile cancer pioneer to take up the concept was Dutch urologist Simon Horenblas. Horenblas and coworkers published a series of publications rediscovering and refining the sentinel node biopsy within penile cancer and uro-oncology. The group in Amsterdam started the first penile cancer studies in 1994 publishing the first results in 2000.^{35,70,71}

The Horenblas Penile Cancer Sentinel Node Protocol of the Early 1990s

The Amsterdam sentinel node protocol of the Horenblas Group only enrolled clinically lymph node negative patients (cN0). In the early 1990s evidence was available that cN0 patients would harbour occult metastatic deposits in 20%-25% of cases.²⁵ This was a true clinical dilemma since a prophylactic inguinal lymph node dissection in all cN0 patients would result in over-treatment of 75%-80% with the possible treatment-related complications associated with inguinal lymph node dissection.²⁵

Resembling the upper extremity morbidity experienced after axillary lymphadenectomy in breast cancer, chronic lower extremity lymphedema after inguinal lymph node dissection in penile cancer is a challenge. Inguinal lymph node dissection is also associated with wound dehiscence, wound infections, lymphoceles, and skin flap necrosis at rates between 30% and 80%.^{28,29,72,73} These conditions can lead to a significant reduction in an otherwise active lifestyle and have a markedly negative impact on patients' postoperative ability; Hence, a reliable, safe and minimally invasive staging method was very attractive.^{38,39,40}

In the first studies by the Horenblas Group, penile cancer patients were intradermally injected with radiotracer (^{99m}Tc nanocolloid) around the tumor and the lymphatic flow was studied dynamically with a gamma camera during the first 20 minutes after injection and static anterior and lateral gamma recordings were obtained for 5 minutes each and repeated after 2 hours.

Location of the primary draining nodes – sentinel nodes – was marked on the skin surface with ink.

The following day the sentinel nodes were surgically identified by the means of a handheld gamma probe, the skin markings and an additional injection of blue dye near the tumor.

Within few minutes the process of blue dye drainage could be macroscopically observed during surgery since dye was immediately drained by local lymphatic vessels. This aided the visual identification of lymph vessels and nodes during surgical excisional biopsy via small inguinal incisions.

Complementary inguinal lymph node dissection was only performed in patients with metastatic deposits detected in the sentinel node at histopathological examination.³⁵

The diagnostic performance of this early sentinel node technique was not ideal with false negative rates at 17%–22%.^{36,74,75} But the Horenblas Group in Amsterdam continued to believe in the concept and further refined and developed the sentinel node biopsy. With time, gained experiences, continuous prospective data collection, persistent analysis, and attempts to optimise protocol the Amsterdam Group improved the sensitivity and decreased the false negative rate to 4.8%.⁷⁶

Protocol Improvements in Amsterdam

Quality improvements in penile cancer sentinel node biopsy protocol implemented by the Horenblas Group in Amsterdam include several factors.

First, the clinical judgement by inguinal palpation was improved by the addition of inguinal ultrasound by an experienced radiologist. Preoperative inguinal ultrasonography was added to the protocol and if indicated ultrasonography-guided fine needle aspiration cytology from suspicious nodes. The consequence of a positive aspiration cytology from an inguinal basin, was a direct ipsilateral inguinal lymph node dissection.

Secondly, histopathological examination of sentinel lymph nodes was intensified by introducing serial sectioning and cyto-keratin immunohistochemistry, making diagnosis of micrometastatic lesions (metastatic deposits <2 mm) more likely.

Thirdly, the radio-tracer silent groins were surgically consequently explored. A strategy of surgical exploration of groins with non-visualized sentinel nodes and more attention during surgery to palpation of the wound was implemented in order to detect lymph nodes blocked by metastatic growth and thus precluding radiotracer uptake.^{36,77} This protocol extension also benefits those patients whose lymphatic pathways might have been interrupted by previous surgical procedures in the inguinal region such as for example vascular procedures or hernia repairs.

Reproducibility of Good Results in Penile Cancer Sentinel Node in the United Kingdom and Denmark

After protocol refinements by the Amsterdam group, sentinel node biopsy was taken up by a number of centers throughout Europe. Other groups also achieved convincing results with the modernised version of the penile cancer sentinel node protocol.⁷⁸⁻⁸³

The Watkin group in London matched the Amsterdam penile cancer sentinel node protocol and presented excellent results both in single institute publications and in a collaborative project.^{78,79,82}

This testified to the reproducibility of the protocol and demonstrated a consistently high diagnostic accuracy in large, specialised centers.

The Danish approach to sentinel node biopsy in penile cancer was an approximation to the Amsterdam protocol, but without the preoperative ultrasound in cN0 patients. A national multicenter study from four smaller centers in Denmark using this approach avowed for a broader applicability presenting a false negative rate before implementation of preoperative imaging at 11.8%.⁸⁰ The false negative rate decreased to 5.6% after centralisation of the procedure to two centers and combining sentinel node biopsy with a preoperative FDG PET/CT.⁸³

Sentinel node biopsy for penile cancer, was included in the EAU guidelines in 2009.⁸⁴

Meta-analysis of the Penile Cancer Sentinel Node Procedure

The performance and safety of penile cancer sentinel node biopsy remains to be evaluated in a randomised study. A meta-analysis summarised the diagnostic accuracy of seventeen studies and found a pooled sensitivity of studies recruiting only clinically lymph node negative patients and using radiotracer and blue dye at 92%.⁸⁵ The pooled sensitivity of all studies was 88% increasing to 90% after exclusion of studies including patients with clinically palpable nodes (cN+). This might reflect the reports of tumor blockage and rerouting of lymphatic drainage in cases of obstructing metastatic deposits. This also highlights an important point about the sentinel node biopsy procedure, the fact that the methodology is best suited for the discovery of smaller metastatic deposits in cN0 patients.⁷⁷

Morbidity Associated With the Sentinel Node Biopsy Procedure

Apart from the very rare casuistic reports on anaphylactic reactions to Technetium nanocolloid injection^{86,87} and Blue Dye injection⁸⁸⁻⁹⁰ the surgery related complications from sentinel node biopsy are significantly fewer than the alternative inguinal lymph node dissection. As mentioned above complication rates from inguinal lymph node dissections are reported in the range 30%–80% many of which are causing



Figure 1 A 68-year old man presenting with a red painless tumor on the inner foreskin, which after removal turned out to be pathologically T1b squamous cell carcinoma. Presenting as cN0 the man underwent bilateral sentinel node biopsy with no metastatic deposits localised in his sentinel nodes (pN0).

complementary procedures and prolong length of stay.^{28,29,72,73} On the other hand, sentinel node biopsy related morbidity is at minor Clavien grades 1-3a and with reported in 5%-13% of patients.^{79,80,83}

Latest Developments in Penile Cancer Sentinel Node – the Hybrid Tracer

The Amsterdam Group has never ceased developing the penile cancer sentinel node protocol and the latest protocol development was the introduction of a hybrid tracer molecule, combining the previously employed ^{99m}technetium nanocolloid and the fluorescent molecule indocyanine green. This requires periodically darkening of the operating room and allows visualization of lymph vessels and lymph nodes with a near infrared camera. The hybrid tracer molecule has shown favourable characteristics compared to blue dye and may replace blue dye.⁹¹⁻⁹⁵

With either indocyanine green or blue dye sentinel node biopsy in penile cancer remains a complex multimodality procedure involving several specialities. The preoperative imaging in the Danish protocol requires access to and



Figure 2 Sentinel node biopsy with the blue dye injection site visible at the proximal end of the penile shaft and the sentinel lymph node just outside of the inguinal incision with visible uptake of blue dye, which along with the acoustic guidance of the gamma probe aids the surgical team to localize the sentinel node.

interpretation of FDG-PET/CT by nuclear medicine physicians and in the Amsterdam protocol requires inguinal ultrasonography by experienced radiologists. The immediate preoperative SPECT-CT or gamma-localisation requires precise tracer-injection and interpretation of nuclear images by nuclear medicine physicians the day before surgery or on the same day in the morning depending on protocol. The radio-tracer- and (fluorescent) dye guided open surgical biopsies by urologists can often be combined with definitive penile tumor surgery and requires experience and regular exposure. The specimen handling and thorough step-sectioning in the

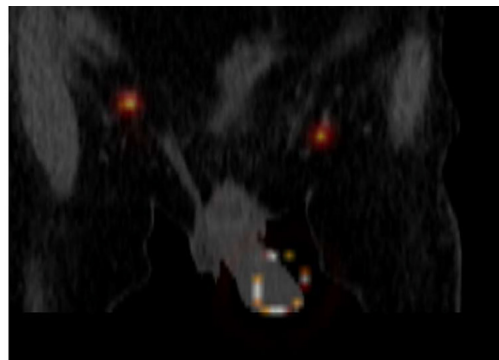
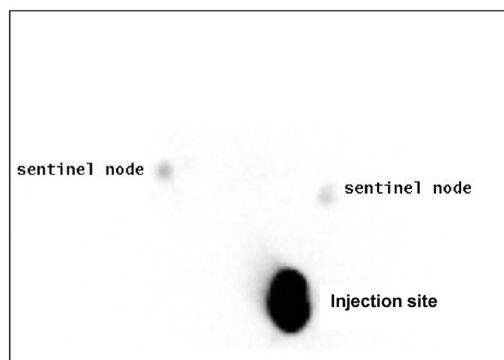


Figure 3 From the same patient on the same day; lymphoscintigram and SPECT-CT images. Injection site and sentinel nodes visualized. Among many sentinel node protocol improvements, the additional anatomical information provided by the SPECT-CT images represent an important leap forward for the preoperative preparation of the surgical team.

Table 1 Table of Historical Contributors to the Understanding of the Lymphatic System

Period	Development	Name (Born-Dead)	Contribution
Prehistory to 1000 BC	"Anatomic Instinct"		
1000 BC-500 BC	Vague Anatomic Speculations		
500 BC-200 BC	Development of Greek humoral theories	Hippocrates (460-370 BC)	Presented a course of disease consistent with cancer metastasizing to lymph nodes. Used the term Karsinos, cancer
	Alexandrian School, human dissections	Herophilus (335-280 BC)	Introduced vessels terminating in ovoid glands in the mesentery
		Herasistratus (304-250 BC)	Mentioned the milky contents of these vessels
200 BC-100 AD	De materia medica	Pedanius Discorides (40 AD-90 AD)	A 5-volume Greek encyclopedia about herbal medicine and related medicinal substances. Suggested herbal extracts for "swollen nodes/glands of the neck."
100 AD-200 AD	Development of galenic theories Roman Law forbids human dissection	Galen of Pergamon (129-199 AD)	Mesenteric lymph nodes and abdominal lacteal vessels containing chyle
200 AD-1400 AD	Byzantine School the Medical Compendium in Seven Books	Paul of Aegina (607-690 AD)	Knowledge of tonsil infections involving neighbouring lymph nodes in the cervical region
1400 AD-1600 AD		Gabriello Fallopio (1523-1562)	Encountered mesenteric "veins" which upon opening spilled "yellow matter"
		Bartholomeus Eustachius (1500-1574)	Documented the milky coloured duct "vena alba thoracis" (ductus thoracicus) in 1552 during a horse dissection
		Ambroise Paré (1510-1590)	Described lymph node involvement in malignancy encountered during surgery
1600 AD-1700 AD		Gasparo Asellius (1581-1626)	1622, the discovery of lymphatic vessels in richly fed dogs
	The idea of circulating blood pumped by the heart	William Harvey (1578-1657)	1628 Publication of Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus, observation of "lacteals"
		Marco Aurelio Severino (1580-1656)	Performed mastectomies with axillary dissection
		Jean Pecquet (1624-1674)	1651, published the anatomic relations of the thoracic duct and its relations to cisterna chyli and the abdominal lymph vessels
		Thomas Bartholin the older (1616-1680)	1653, the first to describe and name the human lymphatic vessels in his work Vasa Lymphatica
	Microanatomic Era	Marcello Malpighi (1628-1689)	Was the first to observe and document capillaries linking the pulmonary arterial circulation and the venous system. Malpighi also described nodal structures lying along the course of the lymphatics solitary and in groups.

Table 1 (Continued)

Period	Development	Name (Born-Dead)	Contribution
1700 AD-1800 AD		Jean Louis Petit (1674-1760)	Demonstrated the spread of mammary cancer to the axillary lymph nodes
		Henri Francois Le Dran (1685-1770)	Demonstrated the spread of cancer along the lymph system in a serial manner
		William Hunter (1718-1783) John Hunter (1728-1793) William Cruikshank (1745-1800)	Demonstration of the progression of fluids through lymph vessels and production of illustrations of lymph vessel anatomy in several species
1800 AD-1900 AD		Thomas Hodgkin (1798-1866)	1832, published an article on malignant enlargement and deformation of lymphoid tissue
		Joseph Pancoast (1805-1882)	1844, reported the first lymphadenectomy in cancer surgery
		Robert Virchow (1821-1902)	1856 published the first in a series of studies focusing on the roles of lymph nodes and the lymph node involvement in cancer
	Physiology Era	Ernest Henry Starling (1866-1927)	Starling proposed that lymph formation is brought about because water is forced out of the capillaries through the pores in the capillary wall by hydrostatic pressure and retained by the osmotic pressure of plasma proteins. The two opposing forces approximately balance but leads to a continuous secretion of fluids into the tissues. The fluid later returns to the circulation via a network of draining lymphatic vessels.
		William Halsted (1852-1922)	1895, published a systematic approach to axillary lymph node dissection and promoted the idea that cancer spreads to the lymph nodes first and from there to visceral organs as a predictable principle
1900 AD-2000 AD	Modern Era	Bernard Fisher (1918-2019)	Modern view of metastatic dissemination of cancer with sequential or simultaneous escape of malignant cells into the lymphatic system and systemic circulation
2000 AD-	Postmodern Era		Circulating Tumor DNA

Table 2 Table of Important Contributors to the Sentinel Node Concept and Sentinel Node in Penile Cancer

Name, Nationality and Specialty (Born-Dead)	Contribution
Anthony Nuck Dutch Physician and Anatomist (1650-1692)	Injected mercury mixed with tin and lead into lymph vessels to visualize them
Marie Philibert C. Sappey French Anatomist (1810-1896)	Published a landmark anatomic study on cutaneous lymphatics using Nuck's method with the injection of heavy metals. His detailed illustrations are still relevant today.
Dimitrie Gerota Romanian Anatomist, Physician, Radiologist and Urologist (1867-1939)	Developed a method for injecting lymphatic vessels with blue dye known in textbooks as the "Gerota method." The blue dye injection enables visualization of lymphatic vessels.
Leonard Ralph Braithwaite British Surgeon (1878-1942)	1923, Used the term "gland sentinel" for omentum nodes draining a bowel segment
Ernest A. Gould American Surgeon (1913-1981)	1960, used the term "sentinel node" in a small study on parotid cancer ($n = 8$)
Ramon M. Cabañas Paraguayan Surgeon	1977 Published a 100-patient lymphangiogram study demonstrating the proposed primary draining lymph nodes, sentinel nodes, in penile cancer
Donald Lee Morton American Surgical Oncologist (1934-2014)	1992, Landmark paper on sentinel node biopsy in melanoma, using blue dye to selectively dissect individual nodes based on individual drainage
Alistair J. Cochran Scottish/ American Pathologist	Published the added value of use of radiotracer and gamma probe for the surgical detection of sentinel nodes in breast cancer in 1993 and oropharyngeal cancer in 1996.
James C. Alex, American Reconstructive Surgeon (1962-2012)	Penile cancer sentinel node revitalization and systematic quality improvements over two decades from 1994, first publication in 2000
David N. Krag American Surgical Oncologist	
Simon Horenblas Dutch Urologist	Large scale external validation of the Horenblas protocol, first publication in 2005
Nicholas Watkin British Urologist	2012 first systematic review and meta-analysis of sentinel node biopsy in penile cancer
Ramin Sadeghi Iranian Nuclear Medicine Physician	

Table 3 Sentinel Node Biopsy Protocol Development in Penile Cancer

	Original Cabañas Protocol 1960s-1970s	Amsterdam Protocol Early 1990s	Amsterdam Protocol 2000-2010	Amsterdam Protocol 2010-	Danish Protocol Before 2010	Danish Protocol After 2010
Sites	1 center	1 center	1 center	1 center	4 centers	2 centers
Early Preoperative Imaging	None	None	Inguinal Ultrasound by experienced Radiologist	Inguinal Ultrasound by experienced Radiologist	None	FDG-PET/CT
Immediate Preoperative Imaging	Lymphangiograms	Gamma camera scintigrams	SPECT-CT	SPECT-CT	Gamma camera scintigrams	SPECT-CT
Operative Procedure: Surgical Excisional Biopsy Guided by	Anatomic Landmarks	Blue dye Gamma signal	Blue dye Gamma signal	indocyanine green fluorescence Gamma signal	Gamma signal some centers: blue dye	Blue dye Gamma signal
Pathology Handling of Lymph Nodes	Hematoxylin eosin bisection	Hematoxylin eosin bisection	Hematoxylin eosin + immunostaining stepsectioning	Hematoxylin eosin + immunostaining stepsectioning	Hematoxylin eosin + immunostaining some stepsectioning	Hematoxylin eosin + immunostaining stepsectioning

pathology department involves immunostaining and accurate lymph node specimen analysis by pathologists with special attention to perinodal metastatic extension. The entire team effort requires well-tested protocols, solid logistics and good collaboration and in rare diseases this calls for centralization of service.²⁴ The sentinel node concept is a minimally invasive, reliable procedure for vital staging of cNO patients and it has a very interesting history (Figs. 1-3, Tables 1-3).

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