Functional Anatomy of the Mediastinal Lymph Nodes in Rats

Michal Pasierbek, MD

Abstract

Background: The lymphatic system remains poorly recognized, yet for oncological reasons, it appears to be of great interest to both scientists and physicians.

Methods and Results: Protocols were performed on 55 adult male Wistar rats. All procedures were conducted after intraperitoneal administration of 4% chloral hydrate and artificial ventilation. The observations of the rats' anatomy of lymphatic structures and observations of lymphatic drainage were conducted after injection of the tracer (ink suspension and/or 1% solution of fluorescein isothiocyanate dextran) into the thoracic wall, intraperitoneally, and into bile ducts, as well as after experimental cutting of the outflow of the lymph from the liver to the mediastinal lymph nodes. The author's own terminology was suggested: medial mediastinal lymph node and lateral mediastinal lymph node. The presence of the tracer was found in the mediastinal nodes after its injection into the posterior wall of the thoracic wall, intraperitoneally, and into bile ducts. After injection into bile ducts, the tracer was observed only in the left mediastinal nodes. After experimental cutting of the basic outflow of the lymph from the liver to the mediastinal nodes, the whole outflow took place through the thoracic duct to the left venous angle.

Conclusions: 1. The locations of the lymph nodes in the thorax as well as the drainage area of those nodes are asymmetrical. 2. Left-sided nodes show larger range of drainage. 3. Cutting the basic lymph outflow from the liver to the mediastinal nodes stops immunological information being transferred to regional nodes of the liver situated in the mediastinum.

Introduction

THE LYMPHATIC SYSTEM REMAINS poorly recognized, yet for oncological reasons, it appears to be of great interest to both scientists and physicians. The literature dedicated to the anatomy of the lymphatic system of rats,^{1–7} the main target of this scientific work, is scarce. Moreover, there is no uniform nomenclature to define the lymphatic system of laboratory animals. Despite the nomenclature, differences between left- and right-sided lymph nodes of the anterior mediastinum are unexplained.^{2,8}

The spread of tumor cells to regional lymph nodes is a universal sign of the progression of many malignant tumors. Metastases to mediastinal lymph nodes are usually related to bad prognosis.⁹ In the 1970s, cutaneous lymphoscintigraphy was developed to determine which regional lymph node is the first to receive the lymph from the tumor. This node was called the Sentinel Lymph Node (SLN). The state of this node indicates the status of all nodes from the regional group of lymph nodes, which allows radical lymphadenectomy to be avoided and proper decisions to be taken as to further treatment.^{10,11}

Knowledge of the anatomy of the lymphatic system, in the context of oncological diagnostics and treatment, is very important. Therefore, this research was performed to describe the mediastinal lymph nodes' anatomy in rats and to verify the drainage area of these nodes in order to identify any alternative routes for the lymph flow after experimental cutting of the main lymph flow from the liver to the thorax.

Materials and Methods

Protocols were performed on 55 adult male Wistar rats weighing 350–400 g. Animals were supplied by the Center for Experimental Medicine of the Medical University of Silesia in Katowice, Poland. Approval of the Local Ethical Committee and permission of the Dean of the Faculty of Medicine in Zabrze were obtained.

All procedures and observations were conducted after intraperitoneal administration of 4% chloral hydrate at a dose of 400 mg/kg body mass and intubation with artificial ventilation with an SAR-830/P Ventilator for little animals (ventilation rate of 70/min and ventilation volume of 2–2.5 mL).

A 1:1 water suspension of drawing ink, with a hydrodynamic diameter of 20–50 nm, and a 1% water solution of fluorescein isothiocyanate dextran 70,000 (FITC-dextran 70,000), with a hydrodynamic diameter of 11 nm, were used in the experiments. Observations of the exposed structures of the lymphatic system used a Nikon SMZ800 stereomicroscope with white light after ink injection and violet-blue light after FITC-dextran injection. Upon completion of all the procedures, euthanasia was performed by cutting the aorta above the diaphragm.

Protocol 1

Observation after intratissial injection of markers into the thoracic wall.

Subgroup 1 (15 rats). After anesthesia, 1 mL of ink suspension was injected into the thoracic wall in the anterior, lateral, and posterior area (5 rats for each area).

Subgroup 2 (15 rats). After anesthesia, 1 mL of FITC-dextran was injected into the thoracic wall in the anterior, lateral, and posterior area (5 rats for each area).

Next, under artificial respiration, the thorax was opened by excision of the anterior wall. Visualized structures of the lymphatic system were observed using the stereomicroscope—for 2 h after FITC-dextran injection and for 4 h after ink injection.

Protocol 2

Observation after intraperitoneal injection of the markers.

Subgroup 1 (5 rats). After anesthesia, 2 mL of ink suspension was injected intraperitoneally.

Subgroup 2 (5 rats). After anesthesia, 2 mL of FITC-dextran was injected intraperitoneally.

In both subgroups, after anesthesia, the marker was administered intraperitoneally by puncture of the left lower quadrant of the abdomen. Next, under artificial respiration, the thorax was opened by excision of the anterior wall. Visualized structures of the lymphatic system were observed using the stereomicroscope—for 90 min after FITC-dextran injection and for 2 h after ink injection.

Protocol 3

Observation after injection of FITC-dextran 70,000 into bile ducts in order to visualize the lymph flow from the liver (5 rats).

After anesthesia, the abdomen was opened by median incision from the point lying 2 cm above the pubic symphysis to the xiphoid process and along the line 5 mm beneath the right vertebral arch in order to ensure better access to the hepatoduodenal ligament. After identification of the anatomical structures, the anterior duodenal wall was cut in order to visualize the duodenal papilla. Next, a catheter was inserted through the papilla to the bile duct and 0.5 mL of FITC-dextran was injected. Then, under artificial respiration, the thorax was opened by excision of the anterior wall. Visualized structures of the lymphatic system were observed using a stereomicroscope for 30 min.

Protocol 4

Observation after injection of FITC-dextran 70,000 into the bile ducts after experimental cutting of the outflow of the lymph from the liver to the mediastinal nodes (5 rats).

After anesthesia, under artificial respiration, the thorax was opened by excision of the anterior wall. Cutting of the lymph outflow from the liver to the mediastinal nodes was performed by ligating, near the diaphragm, the lymphatic vessel passing anteriorly to the pulmonary ligament. Next, the abdomen was opened, and 0.5 mL of FITC-dextran was injected into the bile duct as in Protocol 3. Visualized structures of the lymphatic system were observed using a stereomicroscope for 30 min.

Protocol 5

Control group (5 rats).

The same procedures were performed as in Protocols 1–4, except for the marker injection; 0.9% NaCl was administered instead.

Results

Because of ambiguous terminology referring to the mediastinal lymph nodes in the available literature, the author's own nomenclature was suggested, introducing the notions of the medial mediastinal lymph node and the lateral mediastinal lymph node. The left and right medial mediastinal nodes are situated symmetrically, medially to the corresponding superior vena cava, partly covered by the thymus gland. Both lateral mediastinal lymph nodes are situated laterally and dorsally to the corresponding superior vena cava, whereas the right node is situated more dorsally than the left node (Figs. 1 and 2).

Medial mediastinal lymph nodes of an average size of $5 \times 3 \times 2$ mm were single. In one case, no medial mediastinal nodes were found. Lateral mediastinal lymph nodes of an

FIG. 1. Mediastinal lymph nodes of the rat—schema. (A) Right medial mediastinal lymph node; (B) Left medial mediastinal lymph node; (C) Right lateral mediastinal lymph node; (D) Left lateral mediastinal lymph node; (E) Thymus gland. 1. Right cranial vena cava; 2. Heart. 3. Left cranial vena cava; 4. Aorta; 5. Trachea; 6. Pulmonary trunk; 7. Brachiocephalic trunk; 8. Left common carotid artery; 9. Left subclavian artery. A color figure is available in the online version of this article at www.liebertpub.com/lrb

FIG. 2. Medial lymph nodes after ink injection into the posterior area of the thoracic wall on the right side. 1. Right medial mediastinal lymph node; 2. Left medial mediastinal lymph node; 3. Right lateral mediastinal lymph node (dyed by ink); 4. Left lateral mediastinal lymph node; 5. Right superior vena cava; 6. Thymus. A color figure is available in the online version of this article at www.liebertpub.com/lrb

average size of $4 \times 3 \times 2$ mm were absent in two cases, and in one case double lateral mediastinal nodes were found.

Protocol 1

After ink and FITC-dextran injection into the thoracic wall in the anterior and lateral areas, no presence of the tracer was found in the mediastinal nodes. After ink and FITC-dextran injection into the thoracic wall in the posterior area, on the internal surface of the lateral side of the thoracic wall, the lymphatic vessel was shown heading cranially to the lateral mediastinal lymph node on the side of injection (Fig. 3). In

FIG. 3. Left mediastinal lymph nodes after ink injection into the posterior area of the thoracic wall on the left side. 1. Left medial mediastinal lymph node; 2. Left superior vena cava; 3. Lymphatic vessel leading to left lateral mediastinal lymph node; 4. Left lateral mediastinal lymph node (dyed by ink). A color figure is available in the online version of this article at www.liebertpub.com/lrb

each case, the presence of the tracer was found in the lateral mediastinal lymph node on the side of the marker administration (in one case after FITC-dextran injection on the left side, the presence of the tracer was found in the right lateral mediastinal lymph node—in this case, no presence of the left lateral lymph node was observed). It took 5–7 min for FITC-dextran to be found in the lymph nodes after administration, and 2–4 hours for ink.

Protocol 2

After the markers' administration into the peritoneal cavity, the bilateral diaphragmatic lymphatic plexus was shown. The lymphatic vessel, arising from each plexus, ran on the internal surface of the posterolateral side of the thoracic wall to the lymphatic nodes of the anterior mediastinum (Fig. 4). In one case, an additional lymphatic vessel, running on the internal surface of the sternum to the mediastinum, was found. The presence of the marker in the mediastinal lymph nodes on both sides was found in every case (Figure 5). In subgroup 1, the presence of ink was found in both medial mediastinal nodes in two cases, in both lateral mediastinal nodes in one case, and in all lymph nodes of the anterior mediastinum in two cases. In subgroup 2, the presence of FITC-dextran was found in both medial mediastinal nodes in one rat, in both lateral mediastinal nodes in two rats, and in all lymph nodes of the anterior mediastinum in two rats. The time for moving the tracer through the lymphatic vessels to dye the lymph nodes was 7–8 min for FITC-dextran and 1–2 h for ink.

Protocol 3

After FITC-dextran injection into the common bile duct, the appearance of the lymphatic vessels running in the external tunic of the inferior vena cava was observed. The marker filled the vessels, creating the lymphatic plexus on each side of the superior surface of the diaphragm. The vessels from the left and right plexus joined together in one vessel running in the left pulmonary ligament and then in front of the left pulmonary radix. The vessel finally made its

FIG. 4. Lymphatic vessel with lymphangions leading to the left lateral lymph node after intraperitoneal FITC-dextran injection. A color figure is available in the online version of this article at www.liebertpub.com/lrb

FIG. 5. Medial lymph nodes after intraperitoneal ink injection. **1.** Right medial mediastinal lymph node (dyed by ink); **2.** Thymus; **3.** Left medial mediastinal lymph node (dyed by ink); **4.** Left lateral mediastinal lymph node; **5.** Heart. A color figure is available in the online version of this article at www.liebertpub.com/lrb

way towards the left lymph nodes of the anterior mediastinum. In three cases, the vessel was single and ended in the left medial mediastinal lymph node, but in two cases it branched, ending in both left lymph nodes of the anterior mediastinum (Fig. 6). The time from injecting FITC-dextran to reaching the lymph nodes was 4–5 min.

In no case was the presence of the tracer found in the right mediastinal lymph nodes (Fig. 7).

Protocol 4

After FITC-dextran injection into the common bile duct, after previous experimental cutting of the lymph outflow

FIG. 6. Left mediastinal lymph nodes after FITC-dextran injection into the common bile duct. **1.** Lymphatic vessel leading to the left mediastinal lymph nodes; **2.** Part of the vessel leading to the left medial mediastinal lymph node; **3.** Left medial mediastinal lymph node; **4.** Left lateral mediastinal lymph node; **5.** Part of the vessel leading to the left lateral mediastinal lymph node. A color figure is available in the online version of this article at www.liebertpub.com/lrb

FIG. 7. The schema of the lymph outflow from the liver to the thorax, in unchanged circumstances. The flow follows through the vessel running from the liver to the left mediastinal lymph nodes (*thick arrow*). *Thin arrows* indicate the lymph flow from the liver through the abdominal lymph nodes to the left venous angle. **1.** Right medial and lateral mediastinal lymph nodes; **2.** Left medial and lateral mediastinal lymph nodes; **3.** Lymphatic vessel leading from the liver to the left mediastinal lymph nodes; **4.** Thoracic duct leading from the cisterna chyli to the left venous angle; **5.** Lymphatic vessel leading from the liver to the hepatic lymph node; **6.** Hepatic lymph node; **7.** Lymphatic vessel leading from the hepatic lymph node to the cisterna chyli.

from the liver to the mediastinal nodes, only one lymphatic vessel running from the liver's hilum was observed. This vessel led down to the left, crossing the esophagus, to the abdominal lymph node, which receives only part of the lymph from the liver in unchanged circumstances. From that node, the lymphatic vessel led to cisterna chyli lengthening in the thoracic duct, running on the right side of the thoracic aorta. At the level of the left pulmonic radix, the thoracic duct crossed the posterior surface of the aorta to end in the left venous angle. After the experimental cutting of the vessel running along the left pulmonic ligament, in all cases the flow of the marker only through the thoracic duct to the left venous angle was observed (Fig. 8). The time from administration of FITC-dextran to reaching the venous angle was 8–9 min.

Discussion

Table 1 compares the mediastinal lymph nodes' nomenclature used by individual authors. Because of too general terms or the absence of precise description of the node location, analogous comparison with other scientists is impossible. The most similar nomenclature is that used by Shibata et al.¹²

In the present work, the marker administered intratissially reached the mediastinal lymph nodes only after injection into the posterior area of the thoracic wall. This is at variance with

FIG. 8. The schema of the lymph outflow from the liver to the thorax after surgical cutting of the main route. The whole outflow takes place through the cisterna chyli to the left venous angle. **1.** Right medial and lateral mediastinal lymph nodes; **2.** Left medial and lateral mediastinal lymph nodes; **3.** Lymphatic vessel leading from the liver to the left mediastinal lymph nodes; **4.** Thoracic duct leading from the cisterna chyli to the left venous angle; **5.** Lymphatic vessel leading from the liver to the hepatic lymph node; **6.** Hepatic lymph node; **7.** Lymphatic vessel leading from the hepatic lymph node to the cisterna chyli.

Miotti's data.³ Miotti used the method of intratissial ink injection and observed lymph outflow from the anterolateral area of the thoracic wall to the sternal nodes, from which the lymph flows, according to him, to the mediastinal lymph nodes, but from the posterior area of the thoracic wall the lymph is supposed, according to Miotti, to flow to the paravertebral lymph nodes, which do not have any connection to the mediastinal lymph nodes. In the present study, the markers administered only to the posterior area of the thoracic wall reached the mediastinal nodes, which is similar to data of Hebel and Stromberg,⁷ according to which the lymph drainage from the dorsal area of the thoracic wall follows to the anterior mediastinal nodes. In Miotti's work,³ the whole dosage of the marker was probably stopped appropriately in

the sternal or paravertebral nodes and failed to reach the next nodal station. In the present study, the marker got to the lateral mediastinal lymph node from the posterior area of the thoracic wall on the side of the injection, which suggests that the marker from that area reached the next nodal station, or that the lateral mediastinal lymph node is regional, as is the sentinel lymph node for the posterior area of the thoracic wall. Therefore, the results obtained from the present work suggest a connection (direct or indirect) between the posterior area of the thoracic wall and the lymph nodes of the anterior mediastinum. It is worth noting that Miotti³ used only ink injection, whereas in the present study FITC-dextran administration was also performed. FITC-dextran may reach the next nodal stations because of its small hydrodynamic diameter (11 nm), which continues to confirm connections between the posterior area of the thoracic wall and the lymph nodes of the anterior mediastinum.

The fact of the lymph outflow from the peritoneal cavity to the mediastinal lymph nodes is undisputable. One should be aware of the possibility of transportation of tumorous cells using this route. Shibata et al.¹² describe four ink drainage routes from the peritoneal cavity. The first route is along the internal thoracic arteries, the second follows along the phrenic nerves, and the third by the thoracic lymphatic trunk, running on the internal surface of the posterolateral side of the thoracic wall. All of them lead to the lymph nodes of the anterior mediastinum. The last route leads through the cisterna chyli to the thoracic duct. Ezaki et al.¹³ reports the existence of two main drainage routes from the peritoneal cavity to the mediastinum. First, to the parathymic lymph nodes—anterior (parasternal) lymphatic route—and second, to the mediastinal lymph nodes—posterior lymphatic route. The third, less significant route, follows along the thoracic duct.

Drainage by the lymphatic vessels running on the internal surface of both the sternum and the posterolateral side of the thoracic wall to the mediastinal lymph nodes was observed in the present work, which is similar to the first and third route in the report by Shibata et al.¹² and to the first and second route in Ezaki's data.¹³

Using HSA800 and QDs markers, Parungo et al.¹⁴ observed the uptake of the markers by the mediastinal lymph nodes, as well as by the abdominal nodes to the thoracic duct. After bowel resection, uptake only to the mediastinal nodes was declared. One may come to the conclusion that the lymph reaches the mediastinal nodes from the parietal peritoneum (outflow only from the diaphragmatic parietal peritoneum was proved), whereas the outflow to the thoracic duct takes place from the visceral peritoneum by the abdominal lymph

TABLE 1. COMPARISON OF MEDIASTINAL LYMPH NODE NOMENCLATURE

Present work	Miotti ³	Tilney ² and Takahashi ¹	Hebel ⁷	Ślusarczyk ⁸	Shibata ¹²
Medial mediastinal lymph nodes	Anterior mediastinal lymph nodes	Parathymic lymph nodes	Dorsal group of lymph nodes of lymphocentrum mediastinale	Parathymic lymph nodes	Medial group of anterior mediastinal lymph nodes
Lateral mediastinal lymph nodes		Posterior mediastinal lymph nodes		Anterior mediastinal lymph nodes	Lateral group of anterior mediastinal lymph nodes

nodes. According to Parungo, the visceral peritoneum plays the most significant role in the peritoneal lymphatic drainage. According to Tilney,² lymphatic drainage from the peritoneal cavity to the mediastinum takes place entirely to parathymic lymph nodes (Table 1), whereas Takahashi and Patrick¹ report lymphatic drainage to the parathymic nodes and strictly the left posterior mediastinal node (Table 1). This corresponds only partially to the results obtained from the present study, as it was observed that the outflow from the peritoneal cavity always takes place to the mediastinal lymph nodes on both sides of the mediastinum—both medial mediastinal lymph nodes, either both lateral mediastinal lymph nodes, or to every lymph node of the anterior mediastinum. Shih et al.¹⁵ observed symmetrical localization of the marker in mediastinal lymph nodes after peritoneal administration of Tc-99m albumin nanocolloid. Shibata et al.¹² reports greater inflow to the right thoracic lymphatic trunk than to the left one.

To summarize, the material transported from the peritoneal cavity is captured by bilaterally situated mediastinal lymph nodes as well as by the abdominal lymph nodes. In the present work, and in reports by other authors, the marker's storage was sometimes observed in all mediastinal nodes and sometimes in some of them, yet always bilaterally. Theoretically, many factors may influence which lymphatic route is currently used, for example, the respiratory movements, tension of the abdominal muscles, and the body position. In quadrupeds, forced by gravitation, the peritoneal fluid tends to gather ventrally, and probably this is why the first activated route is along the internal thoracic arteries. It seems that, interspecies, interstrain, or individual differences may play a role.

Because of too large a hydrodynamic diameter of ink (20–50 nm), it fails to leave the bile ducts. From this it follows that for visualization of the lymph outflow from the liver, strictly FITC-dextran 70,000 (of a proper hydrodynamic diameter of 11 nm) was used. It is worth noting that the lymph from the liver flows only to the left-sided lymph nodes of the anterior mediastinum. In the present work, the lymph from the liver reached only the left medial mediastinal lymph node in 60% of cases, and both left-sided nodes of the anterior mediastinum in 40% of cases. In no case was the marker's presence in right-sided nodes observed. In the experimental circumstances, namely after the surgical cutting of the outflow of the lymph from the liver to the mediastinal nodes, the lymph traveled via the abdominal nodes to the cisterna chyli. Thus, there is no alternative route for the outflow from the liver to right-sided lymph nodes of the anterior mediastinum and in no case does the lymph meet those lymph nodes, which is difficult to explain. Probably the specific information necessary for the immune system to keep homeostasis may follow from the liver to the left-sided mediastinal lymph nodes with the lymph inflow.^{8,16,17} This might explain the outflow from the liver to the mediastinal nodes; however, it does not explain the asymmetry of the outflow. This allows for a hypothesis of unequal importance of the right- and left-sided lymph nodes of the anterior mediastinum.

Taking the quoted arguments into consideration, it seems that the lymph nodes situated on both sides of the median plane in the rat's anterior mediastinum do not form any homogenous group, which could then be divided into two—left

and right subgroups. They compose a complex of structures, in some functions differing, yet in other ones their role is identical.

Conclusions

The locations of the lateral mediastinal lymph nodes in the thorax, as well as the drainage area of the lymph nodes located in the anterior mediastinum, are asymmetrical. Left-sided nodes show a larger range of drainage than right-sided ones. Cutting the basic lymph outflow from the liver to the mediastinal nodes stops immunological information being transferred to regional nodes of the liver situated in the mediastinum.

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Address correspondence to:
Michał Pasierbek, MD
Department of Anatomy
Silesian Medical University in Katowice
Jordana 19
41-808 Zabrze
Poland

E-mail: miczhalpasierbek@gmail.com,
mpasierbek@szpital.zabrze.pl