Original research

A comparative study on two different pathological methods to retrieve lymph nodes following gastrectomy

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Abstract

Background: The number of lymph nodes harvested during gastrectomy depends on the extension of lymphadenectomy and the method of lymph node retrieval.

Aim: The objective of this study was to evaluate two methods of lymph node retrieval in specimens of gastric cancer.

Methods: The number of lymph nodes was compared using two different techniques. The technique used in the first group was manual dissection following formalin fixation, and the techniques used in the second group was fat-clearing by acetone.

Results: Both groups were comparable for demographic and pathological variables. The average number of harvested nodes was 19.3 ± 6 for the manual group as compared to 26.1 ± 14 in the acetone group (P = 0.003). The differences in the average number of positive nodes did not reach statistical significance (4.6 compared to 6.9 nodes).

Conclusion: The acetone clearing technique enables the evaluation of a larger number of nodes. An increase, but statistically non significant, number of positive nodes was noted in the acetone group.

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1. Introduction

Although the incidence of gastric adenocarcinoma is declining in the United States and the Western world, this disease still remains the second leading cause of cancer death in both sexes worldwide (736 000 deaths, 9.7% of the total) [1].

It is estimated that around one million new cases of stomach cancer occurred worldwide in 2008 (988 000 cases, 7.8% of the total cancer cases), making it currently the fourth most common malignancy in the world, ranking behind cancers of the lung, breast and colorectum. More than 70% of gastric cancer cases occur in developing countries, and half (of the world total) occurs in Eastern Asia [2,3].

According to the American Cancer Society, the estimated number of newly diagnosed gastric cancer patients and deaths resulting from gastric cancer in the United States in 2012 will be 21 320 and 15 070 respectively [4].

Surgical resection is the only potentially curative therapeutic modality for patients with gastric cancer. The presence of lymph node metastasis is one of the most important prognostic factors [5].

In some centers, specimens are often dissected fresh by the pathologist adjacent to the operating theater, allowing immediate pathologic macroscopic evaluation and preparation for optimal fixation [6]. Gastric cancer, as any other specimen, needs to be dissected by an adequately trained pathologist if accurate staging is to be achieved, taking into account that while lymph node identification and evaluation is time consuming, it is of the utmost importance for accurate staging [7].

2. Material and methods

This retrospective study was conducted with the authorization of our Institutional Review Board (IRB Judgement’s Reference No. 223/13). Data was retrieved from patient charts and pathological reports and a computerized database was created.
Since 2009, we have been using acetone as a fat dissolving solution. Our current study compares the results during the years 2005–2008 using manual dissection to the results during the years 2009–2012 using acetone.

All patients diagnosed with gastric cancer underwent a staging process including total body computed tomography (CT) scanning with selective use of positron emission tomography and computed tomography (PET CT), and tumor markers (CEA, CA 19-9).

From 209 patients diagnosed with gastric cancer, 85 were excluded due to metastatic disease, patient decision or general condition. The remaining 124 patients underwent surgery beginning with diagnostic laparoscopy.

The peritoneal cavity was insufflated to 15 mmHg pressure and two 5 mm trocars were introduced. Any suspicious lesion was biopsied and sent for frozen section, and peritoneal fluid was sent for cytology. An open D2 type gastric resection was performed whenever the tumor was judged resectable. All surgeries were performed by senior residents supervised by two attending surgical oncologists.

The specimens were evaluated using two different methods: 61 were embedded in formalin and compared to 63 gastrectomy specimens placed in acetone for 16 h followed by lymph node isolation.

Statistical analyses were performed at the Department of Statistics of the Tel Aviv University. The Student’s test, and Mann–Whitney rank sum test were used for statistical evaluation and the level of significance was 5%.

3. Results

Both groups were comparable for demographic data, tumor location, type of operation, tumor histology and staging (Table 1).

The average number of harvested nodes per patient in the formalin group was 19.3 compared to 26.1 in the group of patients in the acetone group ($P = 0.003$) (Fig. 1).

The difference in the average number of positive lymph nodes did not reach statistical significance: 4.6 in the formalin group as compared to 6.9 in the acetone group ($P = 0.22$). The proportion of positive lymph nodes in the formalin group was 279/1177 (23%) compared to 430/1668 in the acetone group (25%).

4. Discussion

The number of harvested lymph nodes is considered the best parameter known at present to evaluate the radiality of any surgical procedure for cancer and specifically gastric cancer with its multiple lymph node drainage stations [7]. Surgeons with thorough anatomical knowledge of the various lymph node stations and surgical experience should perform the gastrectomy, and experienced pathologists should examine and count lymph nodes creating standardization of the operative procedure and pathological evaluation. Inadequate lymph node harvesting may contribute to stage migration associated with poorer long-term clinical outcomes resulting from pathological under staging [8–11].

Specifically in cases of gastric cancer, the overall number of harvested lymph nodes depends on which type of lymph node dissection is performed, a D1 or a D2 type, with the expectation of a much lower number of nodes in D1. However, a huge difference in the number of lymph nodes is seen, not only in different studies, but also in different individual patients operated by the same team of surgeons and specimens evaluated by the same team of pathologists [12,13]. This fact should raise the existence of another component of the equation, which is the individual anatomical difference of the number of lymph nodes between different individuals.

The American Joint Committee on Cancer (AJCC) requests a minimum number of 16 harvested lymph nodes in order to precisely stage gastric cancer patients [8].

The Intergroup 0116 trial found that only 10% of patients undergoing gastrectomy in the USA underwent the recommended D2 lymph node dissection, 36% underwent D1 LN dissection, and 54% had inadequate lymph node dissection [14].

Inadequate lymph node staging has also been reported in colon cancer patients, with resultant staging inaccuracies and reported worse survival [6,15].

Peyre et al. [16] showed that in esophageal cancer the number of lymph nodes removed is an independent predictor of survival and to maximize the survival benefit a minimum of 23 regional lymph nodes must be removed.

Some studies dealing with gastric cancer demonstrated improved survival rates for patients who underwent extended dissection, and the recommendation of the Japanese Research Society for the Study of Gastric Cancer is routine performance of a D2 lymph node dissection [11].

Takashi et al. [12] showed that among patients who underwent gastrectomy without any lymph node metastasis, patients with 1–4 examined nodes and those with five to nine examined nodes had a significantly lower survival rate than patients with 10–14 and 15 or more examined nodes.

Only few studies dealing with gastric cancer reported on the relation between DFS and lymph node assessment and showed longer time to recurrence when more lymph nodes were assessed.
Several studies showed a correlation between a high body mass index, proximal tumor location, low hospital or surgeon volume, surgery following neoadjuvant treatment and a low number of harvested nodes [20]. In addition to the surgeon performing a radical lymph node dissection, the number of nodes depends on the thorough examination of the specimen performed by the team of pathologists [7]. Currently, most pathologists obtain lymph nodes for histologic evaluation after their identification by sight and palpation. This method for recovering lymph nodes is termed the "manual method." A second method, termed as 'the lymph node clearing technique', treats the surgical specimen by using different fat dissolving solutions. The result is a translucent mesentry that preserves lymph node structure. Nodes as small as 1.0 mm may be dissected out of the clearing fat. The use of such "clearing solutions" may 'up-stage' some patients because of the detection of additional small involved lymph nodes otherwise not detected by the manual method.

The benefit of using the acetone clearing method has already been proved in colon cancer. Vogel et al. [21] showed an average additional identification of 4.4 lymph nodes when compared to the manual method. Scott et al. [22] showed that fat clearing of the mesocolon or mesorectum should be used when traditional dissection has failed to identify at least 13 nodes and the tumor has been classified as Duke's B. Gehoff and his colleagues investigated the use of acetone in patients following preoperative radio chemotherapy and concluded that the acetone method increased lymph node retrieval three fold compared with manual dissection. In addition this study proved that the acetone method allowed reliable molecular analysis [23]. Only 43% of pathologists in a recent survey reported using fat clearing solutions, such as acetic acid [24].

Our current study using acetone as a fat clearing solution in gastric cancer specimens demonstrates the superiority of this technique over manual dissection of lymph nodes by the identification of a statistically higher number of nodes. The increased number of harvested nodes was translated in our patients into a non significant increase in the number of positive nodes and hence staging of the disease.

In conclusion, acetone as a clearing solution used in our patients resulted in an increase in the overall number of harvested lymph nodes and as a result positive lymph nodes, but the figures were not statistically significant. The differences in the overall number of harvested lymph nodes and positive nodes between different individuals operated and specimens evaluated by the same team of surgeons and pathologists might reflect individual differences.

Further research will be needed to evaluate differences in disease free and overall survival comparing the two groups.

**Ethical approval**

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**Author contribution**

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**Conflict of interest**

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

**What is already known on this topic?**

- The presence of lymph node metastasis is one of the most important prognostic factors.
- The American Joint Committee on Cancer (AJCC) requests a minimum number of 16 harvested lymph nodes in order to precisely stage gastric cancer patients.
- The benefit of using Acetone clearing method has already been proven in colon cancer and is being evaluated in other malignancies such as gastric cancer.

**What this study adds?**

- The increased number of harvested nodes was translated in our patients into a non significant change in the number of positive nodes and hence staging of the disease.
- In our patients, acetone as a clearing solution resulted in an increase in the overall number of harvested lymph nodes and as a result positive lymph nodes.
- Further studies with larger cohort of patients using acetone may lead to better staging and treatment of gastric cancer.

**References**


