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Lymph Node and Bone Marrow Micro-Metastasis in Patients with Esophageal Cancer

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ABSTRACT

Esophageal cancer (EC) is the sixth most common cause of cancer-related mortality globally, due to the high frequency of early metastatic disease. In the past, the presence of metastasis was examined based on histological analysis using hematoxylin and eosin (HE) staining. A high percentage of patients experience recurrence after metastasectomy. Recurrence could be due to the presence of micro-metastasis (MM), which could be detected at sites such as lymph nodes or bone marrow using RT-PCR or immune-histochemical staining. Previous studies have reported inconsistent results with respect to the clinical significance of MM, its impact on survival, and how it is affected by chemotherapy. In this study, we reviewed the (1) clinical significance of MM, (2) methods of detection, (3) sites of occurrence, (4) the mechanism associated with MM, and (5) the role of chemotherapy and radiotherapy in patients with EC.

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Introduction

Esophageal cancer (EC) is the sixth most common cause of cancer-related death, causing more than 500,000 deaths annually (5.3% of global cancer mortality) (1). The 5 years survival for EC patients remains poor (20%) since many patients with EC present at an advanced stage (2). Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) account for more than 90% of EC cases. In the United States 64% of EC cases are diagnosed with EAC, 31% with ESCC, and 5% with other

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carcinomas (3). In Asia, including Iran and China, ESCC is the most common form of EC, accounting for over 90% of cases (4). The most common therapy applied for EC consists of esophagectomy with appropriate lymphadenectomy(5) .

Lymph node metastasis is still the strongest predictor of tumor-related death and overall survival after esophagectomy (6). However, a high percentage of patients experience recurrence despite showing negative results for lymph node metastasis according to routine histopathological examinations, suggesting the presence of occult metastatic cells at the time of treatment (7). Based on the American Joint Committee on Cancer (AJCC) staging system (6th edition), metastasis is categorized as macro-metastasis, micro-metastasis (MM), and isolated tumor cells (ITCs); the categories are respectively defined as metastatic tumor tissue >2.0 mm, metastatic tumor tissue ≤ 2.0mm and >0.2 mm, and metastatic tumor tissue ≤ 0.2 mm (8).

The poor prognostic impact of MM has been shown in different types of cancer, including gastric cancer (9), breast cancer (10), and lung cancer (11). Although most of the previous studies on patients with EC indicate poor prognosis in the presence of MM, some report controversial results (12, 13). Also, the impact of chemotherapy on the incidence of MM is unclear. This review aims to survey the methods of detection (cytology, immunohistochemistry, genetic analyses, and culture techniques), sites of MM (lymph nodes, bone marrow, and blood), mechanism of MM, the impact of MM on patients' prognosis, and the role of chemotherapy and radiotherapy in patients with EC.

Methods for detection of micro-metastasis in esophageal cancer

Cytology

Cytology is an old but useful tool in diagnosing cancer. For example, pleural lavage cytology (PLC) can be used to detect the tumor cells released into the pleural cavity in EC and non–small cell lung cancer (NSCLC) during surgical intervention (14) .

Immunocytochemistry and Immunohistochemistry

Identification of occult epithelial tumor cells in the blood, bone marrow, and lymph nodes is based on the differentiation of cells with dissimilar origins e.g., hematopoietic versus epithelial cells (15) .

Most studies conducted on cancer treatment and the presence of latent MM have used immunohistochemistry/immunocytochemistry or reverse transcriptase–polymerase chain reaction (RT-PCR) to identify external epithelial tumor cells. These methods are highly sensitive and can detect as few as one or two tumor cells among 106 bone marrow mononuclear cells (16, 17). Cytokeratin AE1/AE3 and CAM5.2 have been reported as reliable markers for the detection of MM in lymph nodes (18). Bagheri et al. used both macroscopic and microscopic methods (hematoxylin and eosin (HE) staining and cytokeratin immunohistochemistry (CKIHC), respectively) to detect MM in bone marrow samples taken from 43 patients with EC. They also report that the rate of positivity for bone marrow samples was nine (20.9%) in HE method and 13 (30.2%) in CKIHC method (19).

Genetic Analysis

The genetic analyses for the detection of MM include RT-PCR with multiple markers, detection of carcinoembryonic antigens, and melanoma antigen-3, whose messenger RNAs are exceedingly expressed in EC (20). RT-PCR assay can improve the sensitivity of histopathologic examination (i.e., HE staining) in detecting lymph node metastasis and may be beneficial for intraoperative genetic diagnosis following cervical lymphadenectomy in EC patients (20). PCR can detect 1 to 10 cancer cells among 107 normal nucleated cells in bone marrow aspirate or peripheral blood samples with remarkable specificity and sensitivity (21). Given the unstable nature of mRNA in the extracellular environment, its detection by RT-PCR in tissue or fluid strongly indicates the presence of a tumor (22). In a meta-analysis, Iddings et al. reported that immunohistochemistry detected 179/566 (32%) cases with MM in node-negative patients, while RT-PCR identified 64/173

(37%) cases with MM in node-negative patients (23).

Culture Techniques

Tumor clonogenic assays are another method for detecting MM. This method can identify patients with poor prognosis by evaluating the growth of tumor colonies in vitro (24). Culture techniques are more effective than routine analysis in detecting tumor contamination of bone marrow specimens in patients with breast carcinoma or lymphoma. Culture techniques have also been applied to confirm the viability of MM tumor cells in EC and NSCLC (14). It has been reported that using cell culture with immunohistochemistry may increase the detection rate of bone marrow MM in patients with EC (25).

Mechanism of micro-metastasis

The mechanism of metastasis includes cell detachment from the primary tumor, followed by invasion, embolization, and survival in blood or lymph circulation, and extravasation and multiplication in an organ (26). While previous in-vivo studies show intravasation of metastatic cells into circulation and extravasation to organs, growth, and vascularization of MM and mechanisms of macro-metastasis are not fully investigated (27-29). Previously, it has been shown that circulatory tumor cells (CTC) have an intermediate role between primary tumors and metastatic tumors (30). CTCs are valuable in the diagnosis, prognosis, and treatment of different types of malignancies.

One of the popular hypotheses for cancer recurrence and therapeutic failure is the presence of a rare population of cells called cancer stem cells (31). These cancer stem cells have the capacity for self-renewal and differentiation and are responsible for distant metastasis, cancer recurrence, and chemotherapy and radiotherapy resistance (32). Several in vitro studies were done on these cancer stem cells in EC cell lines. In our previous study, we showed the anti-cancer role of Cerium oxide nanoparticles in EC cancer stem-like cells by elevating antioxidants and reducing oxidants (Figure 1) (33). In addition, Javid et al. presented the anti-cancer effect of Aprepitant, which is usually used as an antiemetic drug after chemotherapy, in EC cancer stem-like cells by promoting caspase-dependent apoptotic cell death and G2/M cell cycle arrest (34). These experimental studies suggest new insights into the clinical application of new biochemical treatments for EC and cancer stem cells.

Sites of Micro-metastasis

Lymph nodes

Lymph node metastasis is an important prognostic factor in patients with EC. Radical lymphadenectomy is often performed for patients with EC to reduce the recurrence rate (35). Also, it is important to rule out regional metastasis in EC patients in order to decide whether neo-adjuvant therapy should also be considered along with surgery (36). Radical lymph node dissection, including extended three-field lymphadenectomy, leads to a higher rate of postoperative complications and in-hospital mortality in comparison to surgical treatment in other gastrointestinal cancers (37, 38). Therefore, less invasive surgery and extensive lymphadenectomy is a promising approach for the detection of lymph node MM in patients with EC. Sentinel node navigation has been suggested as an acceptable approach for patients with cT1 and cN0 EC (39). Conventional histological examinations are not appropriate for the diagnosis of lymph node MM. Consequently, meticulous lymph node assessments using IHC or PCR are required in order to determine the proper treatment strategy .

It has been indicated that using a single histological section leads to an underestimation of MM, so a multiple-slice approach for pathological examination would improve the likelihood of detection (18). A previous study reported the rate of detection for MM in lymph nodes to be 3.8%, 6.3%, and 11.8% in one-, two-, and five-slice sampling, respectively (40). The expertise of examiners is another important factor in detecting MM, and can be the cause of differing results between studies. For instance, Shiozaki et al. report that researchers found MM in 31% of lymph nodes in patients with EC, while

pathologists found only 15%. As a result, diagnoses of MM by researchers had no influence on patients' survival while diagnosis by a pathologist had a significant impact on survival (41) .

Bone marrow

Bone marrow is an ideal site for finding MM due to its rich blood supply and mesenchymal nature, which facilitate the identification of the cytokeratin-positive epithelial deposits (42). Detecting MM in bone marrow concurrent with the primary tumor may indicate a metastatic phenotype and tumor dissemination (43). Bone marrow MM can be detected in different skeletal bones such as ribs and iliac crest. However, in a study by O'Sullivan et al., it was shown that in patients with foregut tumors, the incidence MM in bone marrow samples from ribs was significantly higher than the incidence in samples from iliac crest (88% and 15%, respectively) (44). Bone marrow MM has been previously discussed in the context of other cancers such as breast cancer and gastric cancer (45, 46). The clinical significance of bone marrow MM is unclear and different studies present controversial results. Hence, there is no standard laboratory protocol or indication for MM detection in bone marrow for patients with EC.

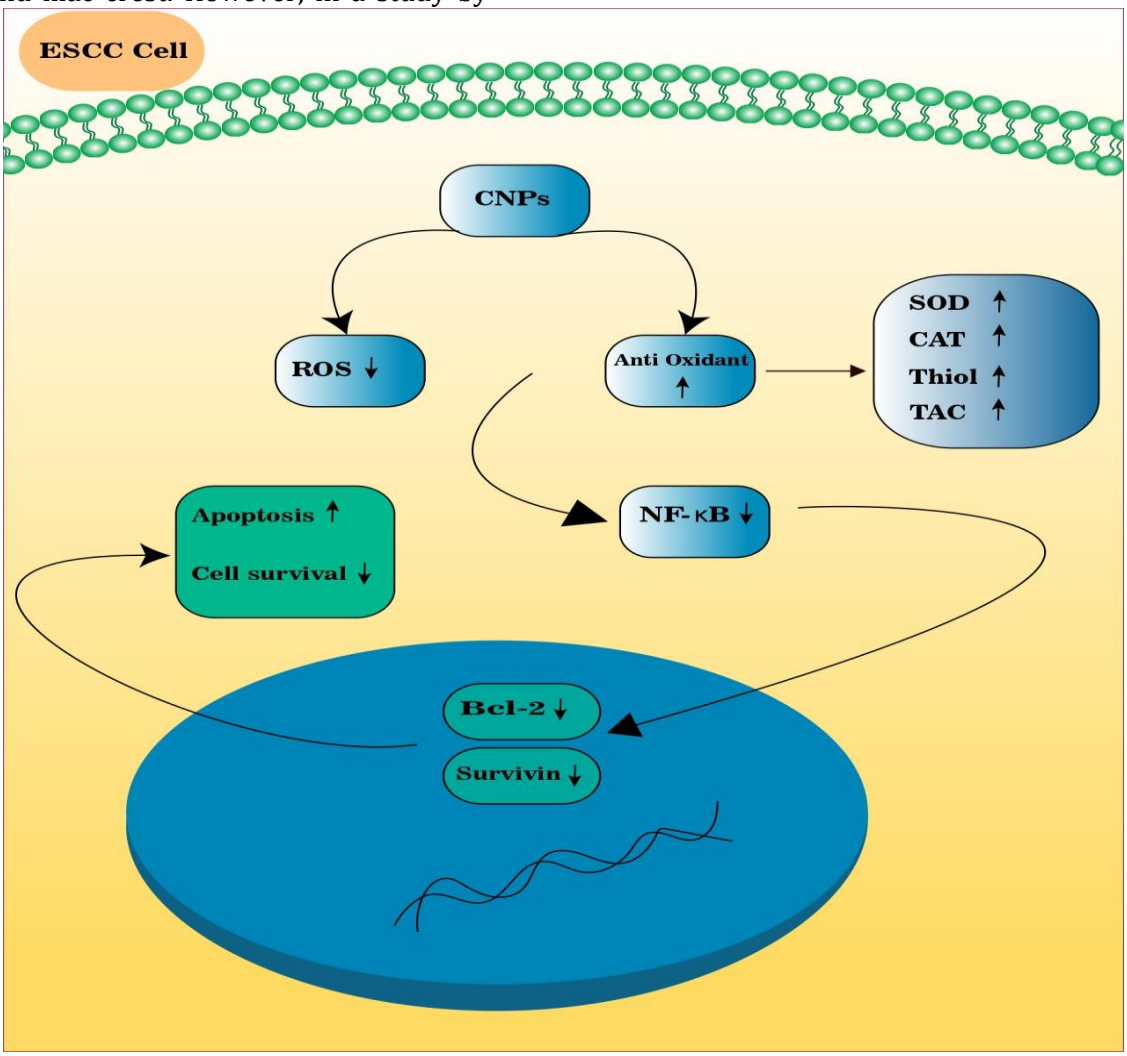

Figure 1. The anti-cancer role of cerium oxide nanoparticles against esophageal squamous cell carcinoma (ESCC) cell line by decreasing ROS (reactive oxygen species) and elevating anti-oxidants including SOD (Superoxide dismutase), CAT (catalase), TAC (total antioxidant capacity), and thiol which lead to downregulating antiapoptotic genes (Bcl-2 and survivin) and inducing cell apoptosis.

Prognosis and clinical significance of micro-metastasis

Examination of lymph nodes and bone marrow by immunohistochemistry or RT-PCR can alter the diagnosis of patients from pN0 to MM positive (47). The presence of MM in lymph nodes or bone marrow is discussed as an important factor of EC recurrence (48). Table 1 summarizes the impact of lymph node MM on survival and prognosis in patients with EC.

Lymph node micro-metastasis

Although MM may be used for breast cancer staging in the current AJCC staging system, it is not included as a staging parameter in EC. Previous studies show contrary results regarding the clinical prognosis of EC patients with MM. Some studies indicate poor outcomes when MM is detected in lymph nodes (5, 7, 12, 47, 49-53), while others do not report any differences in prognosis between EC patients with and without lymph node MM (54-57 ,13).

In a study on 110 pN0 EC patients, Karstens et al. presented lymph node MM and bone marrow MM as independent prognostic factors of overall and recurrence-free survival. They also showed that patients with squamous cell carcinoma and adenocarcinoma did not significantly differ in the rate of MM (47). Moreover, a study by Chen et al. indicated the poor prognosis for EC patients with MM. They also suggest immunohistochemistry as a useful approach for early detection of MM in patients with negative results for the presence of MM in lymph nodes. Furthermore, they suggest that EC patients with IHC-positive staining should be categorized as pN1 (5). Furthermore, the study of Nakamura et al. on 53 pN0 EC patients showed that although lymph node MM may increase the rate of tumor recurrence, it does not affect survival (58). Another study on 50 pN0 EC patients showed 73% and 86% 5-year relapse-free survival in EC patients with or without lymph node MM, respectively (P=0.37) (59). However, Hosch et al. showed the malignant potential and tumorgenicity of tumor cell lines in immune-deficient mice using immunohistochemically-positive but histopathologically-negative lymph nodes. This study indicates the potential malignant features of MM (60).

In summary, most studies on lymph node MM, especially recent studies with large sample sizes, report poor prognosis and survival for EC patients with lymph node MM. This result indicates the role and importance other diagnostic methods in pN0 patients such as immunohistochemistry and RT-PCR.

Bone marrow micro-metastasis

Bone marrow is a common site for tumor involvement for tumors disseminated through the bloodstream (61). Epithelial cell markers do not express in bone marrow, thus detecting epithelial cell markers in bone marrow reveals the presence of metastatic cancer cells (62). However, the clinical importance of bone marrow MM in EC patients is still unclear. Table 2 summarizes the studies on bone marrow MM and indicates the controversial results in different studies. Ryan et al. conducted a study on EC patients with bone marrow MM with a mean follow-up of 10.04 years. The presence of bone marrow MM was examined with IHC. They conclude that bone marrow MM is an independent prognostic factor in EC patients and is correlated with reduced survival (63). On the other hand, Konczalla et al. found no significant correlation between bone marrow MM and disease-free survival in 76 patients in early stages of EC (64). However, most of the studies done on this topic, especially studies with long follow-ups, show a poor prognosis for patients with bone marrow MM (65-67 ,62) .

The discrepancies in the impact of lymph node MM and bone marrow MM can be due to the methods used for MM detection, type of surgery, and type of EC. As noted before, sampling methods have a significant effect on the rate of MM detection in lymph nodes. Also, RT-PCR and immunohistochemistry have different sensitivities in detecting MM. Limited lymphadenectomy can be another factor in the underestimation of MM in lymph nodes. Finally, it has been stated that MM is more common in EAC than ESCC (47).

Concurrent bone marrow and lymph node micro-metastasis

The concurrent presence of MM in bone marrow and lymph nodes of patients with EC is associated with an even poorer prognosis. In fact, a previous study showed patients with EC with both lymph node and bone marrow MM had the shortest overall survival and recurrence-free survival compared to negative bone marrow and lymph node MM, only bone marrow MM, and only lymph node MM. Their results also showed no association between the presence of MM in lymph node and bone marrow. However, the presence of MM in lymph nodes and bone marrow was an independent predictor factor of overall survival and recurrence-free survival (OR= 1.925, CI95%=1.126–3.289 and OR=1.624, CI95%= 1.085–2.431 respectively) (47).

Impact of chemoradiotherapy in patients with micro-metastasis

Randomized clinical trials have shown the effect of surgery plus chemotherapy in improving EC patients' outcomes (68). Table 3 shows the effect of chemotherapy and radiotherapy on the incidence of MM in patients with EC. One of the main goals of chemotherapy in EC patients is to reduce the incidence of lymph node MM and extra-nodal invasion. Yanagi et al., studied 41 EC patients with more than 10 years of follow-up. The patients were randomized into neo-adjuvant chemo-radiotherapy and surgery groups. The researchers found that the group receiving neo-adjuvant chemo-radiotherapy had a significantly lower incidence of MM (69). However, another study indicated that patients who received neo-adjuvant chemotherapy had a similar incidence of MM compared with those who only underwent surgery (70). Another study by Hiraki et al. showed that neo-adjuvant chemotherapy can control MM in advanced EC patients and significantly improve recurrence-free survival. They compared two neo-adjuvant chemotherapy regimens and found that the docetaxel+cisplatin+5-fluorouracil regimen was more effective in preventing MM in patients with EC (71).

Wang et al. compared 20 pN0 EC patients who received neo-adjuvant chemo-radiotherapy with 20 pN0 EC patients who underwent surgery alone. The researchers found a 30% reduction in the incidence of lymph node MM in patients who received neo-adjuvant chemo-radiotherapy. They also report a negative relation between disease-free and overall survival and the presence of lymph node MM in patients receiving neo-adjuvant chemo-radiotherapy (72). Matsuyama et al. stated that testing for MM based on IHC and cytokeratin deposits can effectively indicate the eradication of EC cells by chemotherapy. They also conclude that chemotherapy is more effective in the eradication of lymph node MM than lymph node macro metastasis diagnosed by HE staining (73).

These controversial results can be the result of differences in patient characteristics, chemotherapy and radiotherapy regimens, and surgery techniques. Preoperative chemo-radiotherapy seems to have a clinically significant effect on how MM impacts survival. According to Ryan et al., bone marrow MM was a predictor for significantly poorer prognosis in EC patients who underwent surgery, while its effect was insignificant in EC patients who underwent surgery and chemo-radiotherapy (63).

Conclusion

Figure 2 provides a summary of the present review. EC is one of the most difficult cancers to treat, with a high rate of recurrence and poor prognosis. It commonly metastasizes to lymph nodes, such as cervical, mediastinal, and abdominal lymph nodes. However, a considerable number of patients experience recurrence after lymph node dissection. This can indicate the presence of MM, which cannot be detected by conventional histological examinations. Previous studies have shown the presence of MM in lymph nodes and bone marrow. The method of examination for detecting MM is also an important factor. Immunohistochemistry and PCR showed high sensitivity in detecting MM in patients with EC. Also, a multiple slice approach for pathological examination would improve the likelihood of MM detection in lymph nodes. Although previous studies have shown inconsistent results regarding the impact of MM on patients' survival and its clinical significance in patients with EC,

studies showing poor prognosis appear to be more rigorous and more reliable. Additionally, the location of MM, stage of the tumor, and preoperative chemoradiotherapy seems to have an effect on the prognosis. Studies with large sample sizes, long follow-ups, and a suitable approach for MM detection should be conducted to clarify these discrepancies.

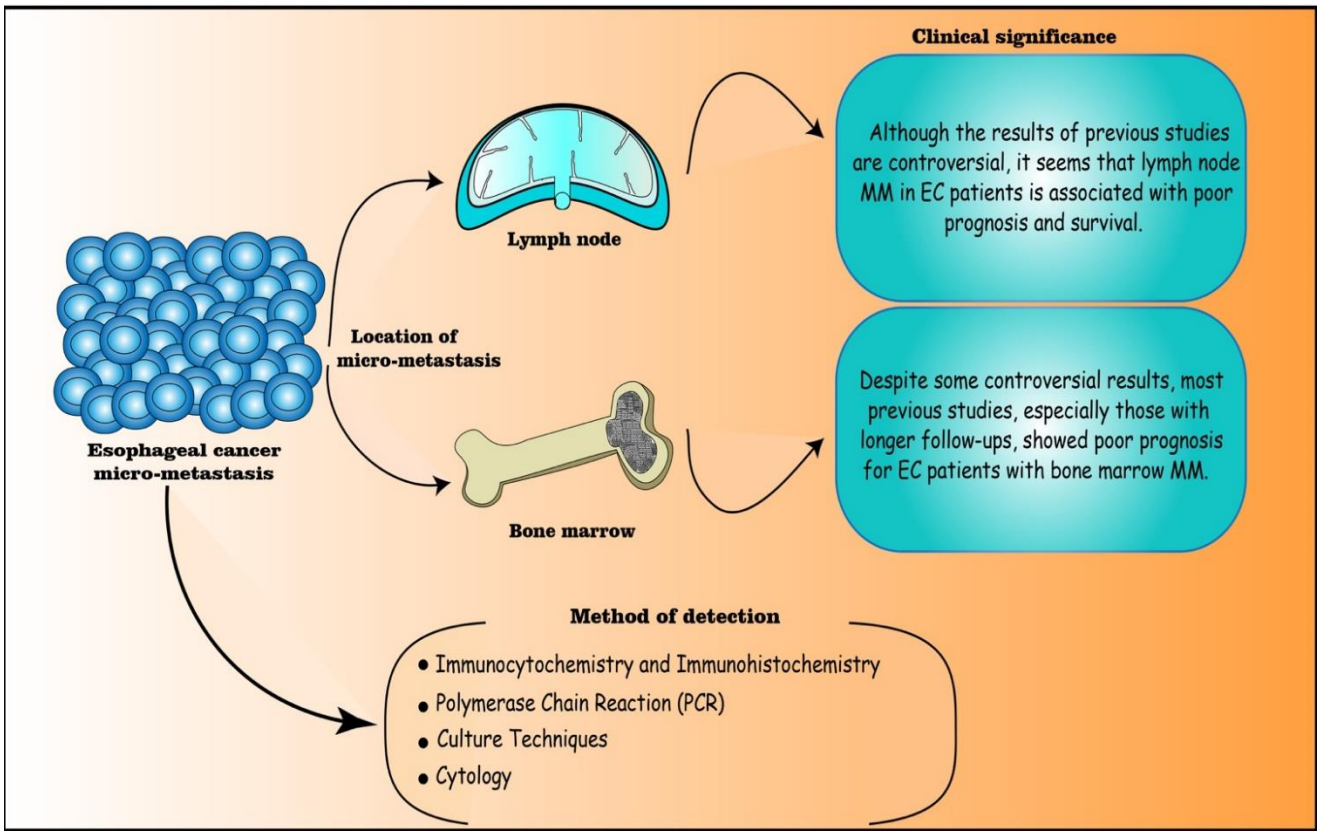

Figure 2. Mechanism, detection, and clinical significance of MM in EC patients.

Table 1. Impact of lymph node Micro-metastasis on survival and prognosis in patients with esophageal cancer.

Author	Year	Method of MM detection	Marker/ antibody	Total Patients(n)	Number of patients with MM	Cancer type	Overall Survival(Positive MM VS negative MM)	p-value	Prognostic significant
Karstens(47)	2020	IHC	AE1/AE3	110	38	Both	--	<0.001	Poor prognosis
Chen(5)	2020	IHC	AE1/AE3	516	88	SCC	(5 years) 53.9% 72.1%	<0.001	Poor prognosis
Yang(49)	2019	IHC	anticytokeratin and mucin 1 antibody cocktail	92	15	SCC	(5 Years) 10% 53.2%	<0.01	Poor prognosis
Prenzel (55)	2012	IHC	AE1/AE3	48	7	Both	(5 Years) 57% 82%	0.002	No prognostic significant
Sun(12)	2010	RT-PCR	MUC1	82	23	SCC	(5 Years) 21.7% 61.7%	0.00	Poor prognosis
Marjanovic (7)	2010	IHC	Cytokeratin antibodies	70	16	Both	(5 Years) 30% 54%	<0.02	Poor prognosis
Li (51)	2007	RT-PCR	MUC1	93	32	SCC	(5 Years) 18.7% 47.6%	0.004	independent prognostic factors.
Heeren (50)	2005	IHC	AE1/AE3	60	18	EAC	--	<0.001	Poor prognosis
Waterman (57)	2004	IHC	AE1, CAM 5.2	28	17	EAC	(5 Years) 70.5% 81.8%	0.49	No prognostic significant
Tanabe (13)	2003	IHC	AE1/AE3	78	31	SCC	--	Not significant	No prognostic significant
Doki (53)	2002	IHC	AE1/AE3	41	11	SCC	(5 years) 28% 79%	0.0188	Poor prognosis
Matsumoto(74)	2000	IHC	cytokeratin (CK) antibody	59	39	SCC	(5 years) 44.6% vs 91%	0.002	Poor prognosis
Komukai (52)	2000	IHC	anti-cytokeratin antibod	37	14	SCC	--	0.042	Poor prognosis
Glickman (56)	1998	IHC	AE1/AE3	145	67	Both	(2 Years) 58% vs 55% for EAC , 80 vs 89 for SCCC	0.46 for adenocarcinoma 0.90 for scc	No prognostic significant

MM: Micro-Metastasis, **SCC:** squamous cell carcinoma, **EAC:** esophageal adenocarcinoma ,**IHC:** immunohistochemical, **RT-PCR:** Reverse transcription polymerase chain reaction

Table 2. impact of bone marrow Micro-metastasis on survival and prognosis in patients with esophageal cancer.

Author	Year	Method of MM detection	Marker/ antibody	Total Patients(n)	Number of patients with MM	Cancer type	Overall Survival (Positive MM VS negative MM)	p-value	Prognostic significant
Karstens(47)	2020	IHC	AE1/AE3	110	54	Both	--	<0.001	Poor prognosis
Konczalla (64)	2019	Immunocytochemical	pan-keratin antibody A45-B/B3	76	13	Both	--	0.455	Not significant
Ryan(63)	2014	IHC	anti-cytokeratin-18	88	47	Both	(10 years) --	0.014	Poor prognosis
Chen(65)	2014	RT-PCR	CK19	61	13	SCC	(5 years) 15.4% vs 59.7	<0.001	independent prognostic factor
Gray (54)	2012	IHC	CAM 5.2 and AE1/AE3	42	19	Both	21.1% vs 21.7%	0.99	Not significant
Zhang(62)	2010	RT-PCR	CK19	61	13	SCC		0.002	independent prognostic factor
Macadam (75)	2003	IHC	antibody Ber-EP4	31	11	Both	(One year) 36% vs 81%	0.03	Poor prognosis
Nakamura(66)	2003	IHC	Anti-cytokeratin	52	13	SCC	--	<0.001	Poor prognosis
Natsugoe (67)	2003	RT-PCR	CEA	48	10	SCC	(4 years) 10% vs 47.3%	--	Poor prognosis
Thorban (76)	2000	IHC	anti-epithelial-cell antibody A45-B/B3	75	29	SCC	--	<0.001	Poor prognosis

MM: Micro-Metastasis, **SCC:** squamous cell carcinoma, **EAC**: esophageal adenocarcinoma, **IHC:** immunohistochemical, **RT-PCR**: Reverse transcription polymerase chain reaction

Table 3. Impact of chemoradiotherapy on the incidence of micrometastasis in patients with esophageal cancer.

Author	Year	Site of MM	Patients(n) chemo or radiotherapy vs surgery alone	Type of esophageal cancer	Method of MM detection	Type of chemo or radiatherapy	Incidence of MM after Chemo or radiotherapy group vs surgery group	P-Value
Yanagi(69)	2018	Lymph node	20 vs 21	SCC	IHC	Radiotherapy + cisplatin+ 5-fluorouracil	30% VS 76.2%	0.003
Wang (72)	2013	Lymph node	20 vs 20	EAC	IHC	Radiotherapy+ paclitaxel+carboplatin	10 % VS 40%	0.028
Matsuyama (73)	2007	Lymph node	75 VS 32	SCC	IHC	Cisplatin + doxorubicin hydrochroride+5-fluorouracil	24% vs 47%	0.19
Natsugoe (70)	2000	Lymph node	20 vs 20	SCC	IHC	cisplatin+ fluorouracil + leucovorin	55% VS 50%	Not significant

MM: Micro-Metastasis, **SCC:** squamous cell carcinoma, **EAC:** esophageal adenocarcinoma, **IHC:** immunohistochemica

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