Iliac crest biopsy versus rib segment resection for the detection of bone marrow isolated tumor cells from lung and esophageal cancer

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Abstract

Objective: The presence of isolated tumor cells in the bone marrow affects the prognosis of both esophageal cancer and non-small cell lung cancer (NSCLC). Therefore, preoperative assessment of isolated tumor cells may be useful to plan multimodality treatment. Rib segment resection at surgery provides adequate amounts of bone marrow for the detection of isolated tumor cells while bone marrow aspirate from the iliac crest does not. The iliac crest biopsy according to the Jamshidi technique procures a core of tissue apt for histology and not simply for cytology. The aim of this study was to compare the accuracy of iliac crest biopsy versus rib segment resection in the diagnosis of isolated tumor cells in order to obtain a useful preoperative approach. Material and methods: Twenty-one consecutive patients (18 NSCLC, three esophageal cancer) were evaluated. None had chemotherapy prior to evaluation. Bone marrow was obtained preoperatively by iliac crest biopsy using the Jamshidi needle and at surgery by rib segment resection. Positive cytokeratin neoplastic cells were searched by immunohistochemistry on tissue sections from the iliac crest biopsies and by flow cytometry on cell suspensions from the rib segments. Results: Isolated tumor cells were detected in the rib segments of ten patients. In all cases the Jamshidi needle biopsy was not diagnostic. Conclusion: Our results suggest that, if the diagnosis of bone marrow isolated tumor cells has clinical relevance, the preoperative assessment should be performed by rib segment resection or methods other than iliac crest aspirate or biopsy. Further investigation is needed to determine whether isolated tumor cells have a preferential spread to chest bones other than distant bone sites. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The current staging system for both non-small cell lung cancer (NSCLC) and esophageal cancer have important limitations as revealed by the variability of survival rates among different series [1–3].

A critical role in this context may be played by the presence of occult isolated tumor cells in lymph nodes and/or bone marrow. The prognostic relevance of micrometastases is gaining evidence in various tumors including NSCLC and esophageal cancer [4–9].

In NSCLC and esophageal cancer occult isolated tumor cells have been investigated in the bone marrow obtained from both iliac crest and rib segments [4–6]. Results have suggested a significantly higher yield of positivities in rib segment bone marrow than in iliac crest bone marrow aspirates at least for esophageal cancer [6]. This finding may be consequent to the quality of the material obtained by iliac crest needle aspirate [6,10].

The iliac crest biopsy according to the Jamshidi technique provides a core of tissue that after appropriate treatment may be used both for morphology and immunohistochemistry [10,11]. This technique should avoid artifacts due to mechanical stress to the cells and possible contamination [10].

The goal of this study was to verify whether iliac crest biopsy might represent a useful tool for the staging of NSCLC and esophageal cancer patients and a more rational application of multimodality treatment protocols [12,13].

2. Material and methods

After giving informed consent, 18 patients affected by non-small cell lung cancer (ten adenocarcinoma and eight...
squamous) and three patients with esophageal cancer (two squamous cancer of the esophagus and one adenocarcinoma of the gastric cardia) underwent preoperative iliac crest biopsy using the Jamshidi needle and rib segment resection at thoracotomy. None of the patients received neoadjuvant chemotherapy prior to bone marrow assessment of both iliac crest biopsy and rib segment.

2.1. Rib bone marrow assessment

The bone marrow of 3-cm long rib segments obtained from open surgery field prior to tumor resection was flushed out with saline solution. Cells were then washed twice with phosphate-buffered saline (PBS) and separated on Lymphoflott (Biestcol, Germany) for 20 min at 400 g, and washed again with PBS. The cell concentration was adjusted to 5 × 10^6/ml. Aliquots of 1 ml were washed and fixed with 70% ethanol. Cells were then washed with PBS and permeabilized with PBS–saponin 0.1%. Cells were incubated for 30 min with fluorescein isothiocyanate-conjugated monoclonal antibody, FITC mAb MNF 116, against cytokeratins 5, 6, 8, 17, and 19 (Dako, Denmark), washed twice with PBS and counterstained for DNA content with propidium iodide containing ribonuclease using the DNA Stain kit (Coulter, Miami, FL). Control samples were run with appropriate isotypic control. Cells were then analyzed using a cell cycle analysis program on an Epics Coulter Elite flow cytometer (EPICS XL, Coulter Electronics, Miami, FL).

2.2. Iliac crest bone marrow assessment

Jamshidi needle biopsies from the posterior iliac crest with a bone marrow tissue cylinder longer than 1.5 cm, were fixed in B5 for 2 h, followed by washing in alcohol at 70°C for 30 min, decalcification in Decal (EDTA bisodic salt) for 2.5 h and ordinary processing with paraffin embedding [11,14]. Three-micrometer-thick serial sections were cut and stained with hematoxylin and eosin, Giemsa and Gomori silver impregnation for reticulin fibers [14]. For immunohistochemistry, further serial sections were cut, coated on naturally charged slides, stored in a warm chamber at 56°C for at least 2 h and then rinsed with water through repeated washes in Bioclear and graded alcohols [11,15]. They were then submitted to optimized antigen retrieval procedures [15] and processed in a Dako TechMate 500, by applying the antibodies MNF116, 34βE12, and 35βH11 (all obtained from Dako), respectively raised against cytokeratins 5, 6, 8, 17, and 19 (MNF116), 1, 5, 10, and 14 (34βE12), and 8 (35βH11). The antibodies were revealed by the APAAP technique [16]. The results were independently evaluated by two observers.

3. Results

3.1. Rib segment bone marrow assessment

Bone marrow obtained from the rib segments resulted positive for isolated tumor cells in 12 patients. All three patients with esophageal cancer (one with stage IIA and two with stage III) had isolated tumor cells within the rib bone marrow. Among 18 patients with NSCLC, seven of ten with adenocarcinoma had cytokeratin-positive cells in the rib bone marrow, while only two of eight with squamous cell cancer turned out to be positive. Table 1 shows the TNM staging of the NSCLC patients.

Table 1

<table>
<thead>
<tr>
<th>Stage</th>
<th>Adenocarcinoma</th>
<th>Rib-ITC</th>
<th>Squamous</th>
<th>Rib-ITC</th>
</tr>
</thead>
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<td>3</td>
<td>0</td>
</tr>
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<tr>
<td>IIB</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

* Rib-ITC, Rib bone marrow isolated tumor cells.

3.2. Iliac crest bone marrow assessment

None of the serial sections obtained from the posterior iliac crest biopsies performed on the posterior iliac crest revealed the presence of isolated tumor cells or clusters both at morphologic and immunohistochemical evaluation.

4. Discussion

The controversial results observed in the ongoing multimodality treatment clinical trials for solid tumors advocate the need for a more detailed staging classification [17–20]. Recent reports have provided evidence on the negative prognostic relevance of occult lymph node and bone marrow isolated tumor cell dissemination both for NSCLC and esophagogastric cancer [4,5,7–9,13]. Some authors have proposed to modify the TNM staging system in order to indicate the presence or absence of regional and systemic occult isolated tumor cells [5,7,9,13]. This supports the International Union Against Cancer (UICC) proposal to introduce the early stage of metastatic disease as category pM1(i) [12].

The presence of isolated tumor cells or clusters has been documented in the bone marrow obtained from iliac crest aspirates and rib segments in both NSCLC and esophagogastric patients [4,6,9]. Interestingly O’Sullivan and colleagues reported in esophagogastric cancer patients a higher incidence of detection of isolated tumor cells in bone marrow obtained from rib segments in comparison with the iliac crest aspirate [6]. His work was the first to investigate such a comparison. Pantel and colleagues, although analyzing bone marrow aspirates from the iliac crest and from ribs, did not investigate such a comparison [4,5]. O’Sullivan et al. propose that the higher incidence of negative results in iliac crest aspirates might be due to the lower
quality of the material and the possibility of mechanical stress on cells [6]. Furthermore, methodological analysis previously reported on immunocytochemical screening for disseminated epithelial cells in bone marrow documented confounding factors when analyzing aspirates [10].

In our study we sought to address the comparison between sites of bone marrow procurement by analyzing bone marrow obtained from rib segments and compare them to iliac crest bone marrow biopsy using the Jamshidi technique. The Jamshidi needle, developed in the 1970s and refined over the years, has proven to be a useful and accurate tool in order to procure 4-cm cores of bone marrow suitable for histology and immunohistochemistry. By this method both morphologic and immunohistochemical evaluations are permitted providing a higher specificity for the search of isolated tumor cell dissemination in the bone marrow [11]. Moreover its sensitivity is regarded to be higher than that of bone marrow aspirates, if the length of the cylinder examined exceeds 1.5–2 cm [21].

In our series immunohistochemistry of the iliac crest biopsy was carried out by using a pool of monoclonal antibodies for different cytokeratin epitopes in order to increase the sensitivity and efficiency of the analysis; nevertheless we did not observe any isolated tumor cell or cluster in the iliac crest bone marrow. By contrast, 12 of 21 patients were positive at flow cytometry assessment of bone marrow from rib segments.

All of the Jamshidi biopsies we studied were longer than 1.5 cm; in addition, in all instances, at least 20 sections were evaluated at different levels and immunohistochemically tested by applying three different monoclonal antibodies against cytokeratins of different molecular weight (wide spectrum cytokeratins, clone MNF116/DAKO; cytokeratin 8 low molecular weight, clone 35BH11/DAKO; cytokeratin high molecular weight, clone 34BE12/DAKO). This is a very efficient antigen retrieval technique, and a highly sensitive detection system [14,15]. In our own and other groups’ experience, such an approach (morphology and phenotyping) allows the detection of single neoplastic cells, as well as the cytological evaluation of immunostained elements [11,15]. The latter point is relevant, since cytokeratin expression can physiologically occur in reactive and neoplastic plasma cells [21]. Thus, it is indeed unlikely that the negativity observed in our biopsies from the iliac crest is due to the low sensitivity of the technique employed.

This study is the first one to investigate in NSCLC the comparison of the presence of isolated tumor cells within the bone marrow procured by biopsy from the iliac crest versus rib segments. Our results are similar to those reported by O’Sullivan and colleagues in esophageal cancer patients, and further sustain the possibility of a role of the site of the primary tumor towards the site of bone marrow isolated tumor cell dissemination [6]. Further investigation is needed to determine whether isolated tumor cells from NSCLC cancer and esophageal cancer have a preferential spread to chest bones other than distant bone sites.

Although yet very limited, the case series we are presenting does not reflect the observation from Pantel and colleagues on a similar frequency between patients with adenocarcinoma and those with squamous cell cancer [4].

From the perspective of isolated tumor cell dissemination in the bone marrow playing a role of independent factor for poor prognosis [4–6,9,13] in NSCLC and esophageal cancer, our results and those reported by O’Sullivan suggest that the search for these entities must be carried out by rib segment bone marrow analysis rather than by iliac crest aspirate or biopsy. This is of great importance from the viewpoint of standardization of novel diagnostic procedures before official acceptance in the TNM classification [12,22].

The impact of bone marrow findings on multimodality treatment is yet to be defined. Isolated tumor cells seem to express tumorigenic activity when in the appropriate environment [6]; although being in a dormant phase within the bone marrow they have a low response to conventional chemotherapy [13,23], but seem to be sensitive to immunotherapy [4,13,24,25].

References


