The Detection of Micrometastases: Is it a Relevant Clinical Parameter?

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The critical observation that secondary carcinoma cells can be found in the peripheral blood was first recorded over a hundred years ago. Since then, attempts have been made to improve the detection of micrometastases from many types of cancers, using histological, immunological and molecular methods. At the same time, other workers were interested in determining whether the occurrence of microscopic deposits of cancer cells had any clinical significance. In particular, considerable research focused on the prognostic impact of micrometastases of carcinomas to bone marrow.

Because of recent technological advances that have provided extremely sensitive methods for detecting small numbers of disseminated cancer cells, it seemed timely to review this important topic. We surveyed the English language medical literature through MEDLINE (macspirs, version 2.4, Silver platter international software), from January 1960 to December 1998 using the following search terms: Bone marrow micrometastases, Carcinoma, Immunocytochemistry, and Prognosis. The principal focus of this brief overview will be on the methodological approaches to demonstrate micrometastases and the prognostic implications of their occurrence.

As early as 1841, Langenbeck [1] suggested that cancer cells from a primary cancer were carried to remote sites by the bloodstream. Somewhat later Thiersch [2] also hypothesized that tumor cells should be able to escape through the venous system into the general circulation. But it was only in 1869 that Ashworth [3] was actually able to demonstrate the presence of carcinoma cells in the peripheral blood. How these cells entered the bloodstream remained an open question. However, it was soon recognized that venous invasion apparently occurred in most solid cancers [4]. The phenomenon was most common in poorly differentiated tumors, where it was frequently associated with distant metastases, and poor prognosis [5–7]. Lymphatic permeation with subsequent metastasis also found support in numerous observations [8–10]. The molecular events associated with permeation of tumor cells into vascular channels are the subject of increasing interest and investigation [11], however this topic is beyond the scope of the present review.

Detection methods

Despite the long period since the recognition of micrometastases, it is only in the past few decades that technical advances have allowed their ready and reliable detection.

While blood-borne cancer cells were traditionally identified according to histological criteria, sensitivity has been greatly enhanced by newer immunological and molecular biotechnology. For instance, paraffin-embedded tissue sections or cytological preparations can be incubated with monoclonal or polyclonal antibodies directed against epithelial-specific antigenic proteins. The expression of some of these proteins is highly specific or even characteristic of certain malignancies. The antibodies, in turn, are detected by immunohistocytochemical or immunofluorescent methods [9,10,12,13]. Cytological methods offer the advantage of concentrating cells by centrifugation. From these cell concentrates a smear is prepared that can be similarly examined for tumor cell antigens [12].

In 1980, Sloane et al. [14] first reported the successful application of immunocytochemical methods for the detection of disseminated carcinoma cells in bone marrow aspirates of breast cancer patients. In that report they introduced the term "micrometastatic cells" [15]. Since then, immunocytochemical methods have been used by many investigators to detect tumor cells in the peripherial blood [16], bone marrow [17,18] and lymph nodes [9,10,12,19].

A new alternative is the reverse transcription-polymerase chain reaction, which is the most widely used molecular method for detecting micrometastases [20]. Cells are derived from blood, bone marrow or lymph node by cytocentrifugation techniques, and their RNA is extracted. Using a reverse transcriptase enzyme, the RNA is then converted into cDNA. These submicroscopic deposits of tumor cell DNA can then be amplified (i.e., copied) hundreds of thousands of times by means of PCR. Since the amplified product contains such a high concentration of the tumor DNA it is feasible to visualize and identify this genetic material using other standard methods for studying DNA.

PCR = polymerase chain reaction

The typical procedure involves gel electrophoresis of the PCR product. The latter forms a discrete band on the gel that is detected by a fluorescent DNA stain, such as ethidium bromide. The tumor DNA in the band can then be further characterized by sequencing the bases in the amplified DNA fragment [12]. As with any highly sensitive technique, the issue of specificity is crucial and in some areas is still debated [20].

Despite the popularity of the new technology, traditional pathological methods still have a place in the search for micrometastasis. In a review of 2,400 patients with breast cancer, Munro-Neville [21] evaluated multiple microscopic sections of each tissue block. He detected lymph node metastases in an additional 13% of cases that seemingly were free of metastatic diseases based on histological examination of a single level. Thus, a number of methods currently exist to enable rapid and reliable detection of microscopic deposits of secondary cancer cells in both blood and tissues.

Micrometastases as a prognostic factor

The ability to reliably detect small numbers of disseminated cancer cells has raised the second serious issue, namely, the potential clinical significance of these micrometastases [22]. The main focus has been on micro-deposits in bone marrow (see below) and lymph nodes [8,10]. The significance of lymph node micrometastases might be even greater today in the era of sentinel lymph node biopsy of breast cancer and melanoma. The presence of bone marrow micrometastases, as detected by immunological methods, was found to be associated with an increased risk of recurrent cancer of the breast [7,23,24], lung [25], stomach [26] and colon [27]. Nevertheless, the question remains open. A recent multi-institutional study found that in breast cancer, micrometastases in bone marrow was not associated with loco-regional relapse [13]. Many researchers, using immunological methods, have emphasized the importance of quantifying the number of metastases in the bone marrow aspirate [8,28]. The number of micrometastases has been found to be a significant predictor for recurrent disease [23,24,29]. Stage II or III of breast cancers are associated with a prolonged disease-free survival when only a few micrometastases are detected [12].

A statistical overview of a segment of this literature utilized a meta-analysis of studies on bone marrow micrometastases [15]. The analysis included 20 previous studies with a total of 2,494 patients. A prevalence of 35% positive bone marrow micrometastases was found in breast cancer (range 2–48%), and 36.5% (range 26.9–59.7%) in cancers of the gastrointestinal tract, head, neck and lung [15]. Moreover, in breast cancer, the presence of bone marrow metastases was highly correlated with involvement of axillary lymph nodes [30]. Regarding the prognostic significance of micrometastases in the bone marrow, most of the studies showed a direct association between the presence of bone marrow micrometastases and a short relapse-free interval [15]. On the other hand, the occurrence of *circulating tumor cells* does not appear to be a reliable indication of disease behavior with regard to predicting disease recurrence. But it still may be clinically valuable to uncover circulating cancer cells as a basis to predict the likelihood of distant metastases, as found for prostatic carcinoma [16].

It appears also from other reports [23,24,29] that the presence of *bone marrow micrometastases* is an important factor in defining tumor stage. Consequently, it has been recommended that this parameter should be included as a facultative prognostic factor in the TNM (tumor-node-metastases) classification [15,31]. On the other hand, it is important to bear in mind that the absence of bone marrow metastasis may merely reflect the individualized biology of particular tumor cells, rather than a less aggressive behavior of the particular tumor. For instance, the intrinsic biologic characteristics of micrometastatic cells apparently determine the preferential bone localization of metastatic deposits from breast and lung cancer, and the infrequency of such an event among gastrointestinal tumors [32,33].

Despite the impression of their clinical relevance, it is still not clear whether micrometastases are an independent prognostic variable [15]. Investigating this issue, Komukai et al. [10] recently reported that immunohistochemically demonstrated micrometastases to lymph nodes in esophageal cancer have an independent prognostic importance for relapse-free survival as determined by multivariate analysis. More of this type of statistical treatment is clearly needed in future studies. For breast cancer the situation continues to be controversial. Diel et al. [33] showed in their study – which included the largest cohort of breast cancer patients – that the prognostic impact of positive bone marrow micrometastases overcomes that of the nodal status. However, others have not found evidence that bone marrow deposits represent an independent prognostic factor as compared to progesterone receptor expression or lymph node status [15].

Implications for the future

As suggested above, a number of basic issues still need to be clarified before the clinical utility of micrometastatic deposits can be properly understood. Micrometastatic disease is not a simple, uni-dimensional entity. Consequently, the intrinsic variables defining this phenomenon – such as metastatic site, tumor load, tumor type, site of origin, previous therapy – still need to be clearly established within a common comparative framework. Some of the observations in the literature need to be repeated with larger cohorts as well as in relation to the broad array of therapeutic options currently available. Appropriate statistical models should be employed to identify the situations in which micrometastatic disease is an independent prognostic variable. According to the recommendations of the International Cancer Committee, evaluation of a new prognostic factor requires a sufficient number of patients to avoid

premature judgments derived from statistical errors [34].

In concrete terms, does this finding have any impact on the surgical approach and extent of surgery? Should we be more aggressive or less aggressive? It has already been suggested that bone marrow micrometastases in patients with breast or lung cancers might be used for the selection of patients for a more aggressive therapeutic approach [32,35]. Further critical assessment of this concept is still needed. In surgical terms, for example, we need to establish the relevance of bone marrow micrometastases in the decision to perform an axillary lymph node dissection in breast cancer.

The technical approaches for detecting micrometastasis represent an additional nidus of concern in our attempt to clarify the potential place of this phenomenon in the clinical assessment of the cancer patient. Currently, the methodological variability between studies – whether immunological or molecular biological – often makes it difficult to compare results [20,36]. There is clearly a need for the setting of technical standards for the assessment of micrometastasis.

In conclusion, this review has suggested that the application of immunological and molecular methods for the detection of micrometastases has two major implications. We have substantially increased our understanding of the epidemiology and biology of this phenomenon. Additionally, these observations have generally supported the view that detecting micrometastases may be relevant to predicting tumor biology in a given patient. In some instances, the occurrence of secondary microscopic deposits of cancer has already been accepted as an important parameter in assigning therapeutic options. However, before this phenomenon can be considered one of the cornerstones of our workup of the cancer patient, a number of basic technological and prognostic issues still remain to be resolved. Moreover, considerably more data are still required on micrometastatic disease that would allow direct comparisons between studies, together with rigorous statistical treatment.

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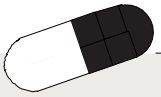
Capsule



Arthritis – another treatment?

The beneficial effects of taking marijuana for debilitating diseases such as multiple sclerosis remains a vigorously debated issue. Evidence for the ability of some constituents of marijuana to influence the immune system, however, does exist. In fact, it is to a non-psychoactive component of cannabidiol (CBD) that some of these effects can be attributed; CBD has been reported to show the down-regulation of lymphocyte and macrophage function.

Extending these studies, Malfait et al. report that oral administration or injection of CBD significantly reduced inflammation in a mouse model of joint disease bearing a similarity to human rheumatoid arthritis. To this end, the cannabidiol appeared to evoke its anti-arthritic effects in two ways: by inhibiting T cell responses normally induced in the arthritis model upon priming with collagen, and through a direct effect on the inflammatory pathways that lead to the eventual damage of joint tissue. Although the mechanisms by which CBD influences the immune system remain obscure, this study may open up new avenues in the search for effective treatment of chronic inflammatory disease.

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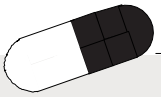
If a tree falls in a forest, and no one is there to hear it, it makes no sound.

George Berkeley, Irish philosopher and Anglican bishop (1685–1753)

Capsule



Parkinson disease and dietary caffeine

Data were analyzed from 30 years of follow-up of 8,004 Japanese-American men (aged 45–68 years) enrolled in the prospective longitudinal Honolulu Heart Program between 1965 and 1968. Incident Parkinson disease (PD), by amount of coffee intake (measured at study enrollment and 6 year follow-up), and by total dietary caffeine intake (measured at enrollment).

During follow-up, 102 men were identified as having PD. Age-adjusted incidence of PD declined consistently with increased amounts of coffee intake, from 10.4 per 10,000 person-years in men who drank no coffee to 1.9 per 10,000 person-years in men who drank at least 28 oz/day. Similar relationships were observed with total caffeine intake and caffeine from non-coffee sources. Consumption of increasing amounts of coffee was also associated with lower risk of PD in men who were never, past, and current smokers at baseline. Other nutrients in coffee, including niacin, were unrelated to PD incidence. The relationship between caffeine and PD was unaltered by intake of milk and sugar.

The findings indicate that higher coffee and caffeine intake is associated with *a significantly lower incidence of PD*. This effect appears to be independent of smoking. The data suggest that the mechanism is related to caffeine intake and not to other nutrients contained in coffee.

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